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Research article

Proximate composition, microbiological quality and presence of total aflatoxins and aflatoxin B_1 in the flesh of three snails' species (*Achatina achatina, Achatina fulica* and *Archachatina marginata*) from a selected locality of Yaoundé, Cameroon

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ABSTRACT

The increasing need for animal proteins has led to an interest in non-conventional protein sources such as snails. Although several species of snails are locally reared and highly prized by Cameroonians, there is a lack of information regarding their composition and safety. This work aimed at assessing the chemical composition, the microbiological quality and the total aflatoxins (AFs) and aflatoxin B_1 (AFB_1) contents of the fleshes from three snails' species traditionally reared in the city of Yaoundé, Cameroon. Samples of *Achatina achatina* (10), *Achatina fulica* (10) and *Archachatina marginata* (10) were randomly collected from a selected farm in Mimboman quarter of Yaoundé and their chemical composition and microbiological quality were evaluated through AOAC and ISO methods, respectively. Their levels of AFs and AFB_1 were assessed using competitive ELISA. The results showed that snail fleshes were a good source of proteins and iron with the one from *A. achatina* containing the highest protein (15.26%) and iron (7.80 mg/100g) contents. Microbiological analyses revealed that the total aerobic counts of the different samples of snail fleshes were all higher than 6 Log CFU/g thus suggesting a reduced shelf life of the raw product. The safety issue of the snail fleshes is questionable as they contained pathogens such as coliforms and *Staphylococcus* spp. at levels higher than the norms. Although yeasts and moulds were found in snail fleshes at loads ranging from 3.5 to 4.17 Log CFU/g, their AFs and AFB_1 contents were respectively below 0.22 and 0.44 ppb, values that are lower than that of raw food intended for human consumption. This study demonstrated the potential of snails as an alternative protein source from animal origin and suggests that particular attention should be paid by the government to sensitize the farmers on good hygiene and farming practices and the consumers on good cooking practices.

1. Introduction

Snail meat is becoming nowadays an alternative source of proteins from animal origin. It is highly consumed worldwide and the report of the Food and Agriculture Organization revealed that in 2017, 18,331 tons of snail meats were consumed in the world (FAO, 2019). The reasons are the high cost of conventional animal protein (beef, fish, pig, poultry, goat, etc.), and the health concerns associated with their consumption (Omole et al., 2006). Opposite to these animal proteins, snail meat is rich in proteins of good quality (with all essential amino acids), polyunsaturated fatty acids and several minerals including zinc and iron (USDA, 2006). The presence of these nutritious and bioactive compounds confers to snail meat potential health benefits (Miegoue et al., 2019). The world markets of snails are dominated by two families. The Helicidae family that accounts for about 70–85% of the global snail market and Achatinidae that accounts for about 15–30%. The most studied snails are from the Helicidae family. That snail family is mainly found in Europe. African species of snails belong to the family Achatinidae.

In Africa, the sector of snails is not well developed. Wild snails represent the great proportion of snails consumed by the population (Miegoue et al., 2019). Snail meat is prized by the African population. Its demand increases every day as it constitutes an important source of

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income for people living in rural rainforest and savanna derivative areas in West and Central Africa. They are collected during the rainy season from their natural habitat which are tropical forests, savannahs, farms, and often gardens where they have unlimited vegetation to feed on (Ngenwi et al., 2010). To satisfy the increasing demands of consumers, immature snails are often collected in both protected and unprotected areas (Cobbinah et al., 2008; Fagbuaro, 2015; Ndah et al., 2017; Miegoue et al., 2019). Associated with deforestation and poor agricultural practices (such as the use of agrochemicals, slash and burn, bush fire, etc.) it might lead to the depletion of the wild snails' population. The consequences are an increase of the snail meat price and environmental modifications because snails also play an important role in nature such as the decomposition of organic matter, the nutrient cycles, and the regulation of microbial activities (Ogogo et al., 2011; Ndah et al., 2017). Besides, the meat from wild snails might be contaminated as the result of their close contact with soil and their uncontrolled feeding pattern. Hence, there is an urgent need to ensure sustainable production of snails. In this light, snail rearing generally called heliculture was introduced to supply snails to meet the consumers' demands and as a wealth-creating opportunity. The *Achatinidae* snails reared in Africa and particularly in Cameroon are *Achatina achatina, Archachatina marginata* and *Achatina fulica*. However, the rearing processes are mainly traditional (Ndah et al., 2017). In most cases, the suitable species of snail is collected in forest and introduced in pens constructed within the backyard of a house (Ejidike, 2002). Snails are fed with green leaves (leaves from paw-paw, sweet potatoes, plantain, cassava, etc.), fruits (banana, avocado, mangoes, etc.) and tubers (Ndah et al., 2017). In these conditions, the newly created environment can modify the proximate composition of snail meat as well as its sanitary quality. It was reported in the literature that the proximate composition of snail meat is significantly influenced by factors such as environment, soil and modifications of the eating habits (Musa et al., 2018). Based on this observation, we hypothesize a modification of the safety issue of snail meat traditionally reared in Cameroon as a result of the change in the feeding pattern. Studies performed in Cameroon regarding snail meat concerned only their domestication and their large-scale production (Ndah et al., 2017; Kaldjob et al., 2019; Miegoue et al., 2019). To the best of our knowledge, none of these studies deals with their microbiological quality and their possible contamination with microbial toxins such as mycotoxins. Hence, the present project was designed and aimed at assessing the chemical composition, the microbiological quality and the contents of total aflatoxins (AFs) and aflatoxin B1 (AFB_1) of the flesh and feed of three species of snails traditionally reared in the city of Yaoundé, Centre Region of Cameroon.

2. Materials and methods

2.1. Study site and period

This study was conducted from March 2019 to July 2019 in Mimboman (3° 5′ N and 11° 31′ E), a quarter located in the 4th district of Yaoundé, Centre Region, Cameroon. The site was chosen as the representative farm for traditional snail rearing because after preliminary investigations it was the only farm in the city where several species of snails were farmed and were daily available. It has an area of approximately 100 m^2.

2.2. Snail specimens

Three species of snails *Achatina achatina*, *Achatina fulica* and *Archachatina marginata* were selected for this study. They represented the main farmed species in Cameroon because of their high productivity and their consumption rate by the local inhabitants. Figure 1 presents the three selected snail species. These identified species were provided to farmers by the Institute of Agricultural Research for Development (IRAD) of Yaoundé, Cameroon.

2.3. Sampling

The samples collection procedure was performed following the method of the European Commission (EC, 2006). Briefly, early in the morning (between 7:30 and 8:00 AM), 10 snails per species were collected at different points of the farm using a systematic random

Figure 1. Three species of snails used in this study.

sampling technique. They were aged approximately 3 months. Visual characteristics of the different species of snails as depicted in Figure 1 were used to select snail samples with similar traits. The 10 samples collected per species were transferred into sterile bags, labelled and transported to the laboratory in an icebox containing frozen blocks.

Besides, 1 kg of the main food used to feed snails (mixture of soya beans and corn flours) was also collected from different parts of the storage bag and conveyed to the laboratory for analyses.

2.4. Samples processing

Upon arrival at the laboratory, under sterile conditions, the snails were washed extensively with distilled water to remove contaminants present at the surface of their shells, rinsed with sterile distilled water and disinfected with ethanol 70% (v/v). Then, the snails were removed from their shells, carefully eviscerated, washed with lemon to remove the slime and rinsed thoroughly with sterile distilled water. Some morphometric parameters of the different snail species were measured. These parameters included the size of the shell, the total weight, the shell and flesh weights. For each species, the fleshes obtained (Figure 2) were pooled and crushed (Black & Decker®, England). The paste obtained was carefully homogenized divided into 3 batches of 50 g. The first batch was directly submitted to microbiological analysis while the two others were kept at -20 °C for physicochemical and mycotoxins analyses. The feed sample was ground and stored for analysis.

2.5. Microbiological analyses of snails

For microbiological analyses, the microbiota searched were the total aerobic mesophilic flora (TAMF), *Staphylococcus* spp., yeasts and moulds, total coliforms, fecal coliforms, *Escherichia coli*, sulfite-reducing *Clostridium* and *Salmonella* spp.

2.5.1. Preparation of stock solutions

The method NF EN ISO 6887-2 (2017) was used to prepare a stock solution of samples. From the 50 g of sample previously ground, 25 g were taken and introduced in a sterile flask containing 225 mL of sterile alkaline peptone water with 2% NaCl. The mixture was homogenized, left on the bench for 30 min at room temperature and serially diluted (10^{-2} to 10^{-6}).

2.5.2. Inoculation procedure and culture conditions

The enumeration of total mesophilic aerobic flora was performed on Plate Count Agar (PCA, LiofilChem, Italy) according to the pour plate method ISO 4833-1 (2013). 1 mL of the different dilutions was added into Petri dish followed by the addition of 15 mL of sterile PCA. The mixture was homogenized, left for gelification and incubated at 30 °C for 48 h under aerobic conditions.

Total and fecal coliforms were enumerated on MacConkey agar (MC, LiofilChem, Italy) (ISO 4832, 2006). An aliquot (0.1 mL) of the different dilutions was surface inoculated onto Petri dishes containing 15 mL of sterile MacConkey agar and spread. The Petri dishes were incubated aerobically at 37°C/48 h for total coliforms and 44°C/48 h for fecal coliforms. Milky white colonies appearing on the Petri dishes were considered as coliforms.

Escherichia coli was enumerated on Eosin Methylene Blue agar (EMB, LiofilChem, Italy) following the method ISO 4832 (2006). 0.1 mL of the different dilutions was spread at the surface of EMB agar and the Petri dishes were incubated under aerobic conditions at 44 °C for 48 h. Metal green colonies appearing on EMB agar were considered as *E. coli*. For confirmative tests, they were submitted to Gram staining, catalase, methyl red, indole, Voges-Proskauer, culture on triple sugars iron agar, dextrose, maltose, lactose, sucrose and mannitol.

Staphylococcus spp. count of the different samples was assessed through the method ISO 6888-2 (1999). 0.1 mL of the different dilutions was spread on Mannitol Salt Agar (MSA, LiofilChem, Italy) followed with incubation at 37 °C for 48 h.

The method ISO 7937 (2004) was used for the enumeration of sulfite-reducing *Clostridium*. Briefly, 2 mL of the dilutions (10^{-1} and 10^{-2}) were introduced into a tube containing 15 mL of sterile Tryptone Sulfite Neomycin agar (TSN, LiofilChem, Italy). The mixture was homogenized and heated at 80 °C for 10 min. Then, the tubes were rapidly cooled and 1mL of sterile paraffin oil was introduced into each tube followed by incubation at 37 °C for 48 h. After incubation, uncolored colonies with black centers were considered as anaerobic sulfite-reducing *Clostridium*.

The enumeration of yeast and moulds was performed by spreading 0.1 mL of the different dilutions onto Petri dishes containing 15 mL of sterile Sabouraud agar supplemented with chloramphenicol (SAB, LiofilChem, Italy) followed with incubation under aerobic conditions at 25°C for 3–5 days (ISO 21527-1, 2008).

The presence of *Salmonella* spp. in samples was assessed according to the method ISO 6579-1 (2017). 25 g of sample was mixed with 225 mL of sterile peptone water and the mixture was incubated for 16 h at 37 °C. Then, 1 mL of the suspension was transferred into a tube containing 10 mL of sterile selenite cystine broth (LiofilChem, Italy) and incubated for 24 h at 37 °C for enrichment. Thereafter, one loopful of each enrichment broth was streaked onto Salmonella and Shigella agar (SS, LiofilChem,

Figure 2. Three species of snails free of shells and slimes.

Italy) agar and incubated at 37 °C for 24 h. Uncolored colonies with black centers appearing on the Petri dish after incubation were considered as *Salmonella* spp. Some microscopic (Gram staining) and biochemical tests (catalase, sugar fermentation, methyl red, indole, and Voges-Proskauer) were performed on presumptive colonies for confirmation.

2.5.3. Plates reading

The colony-forming units (CFU) appearing on the Petri dishes after the incubation period were counted and the results were expressed as colony-forming units per gram of fresh snails (CFU/g). Only plates with colony-forming units between 30 and 300 were considered.

2.6. Determination of total aflatoxins (AFs) and aflatoxin B1 (AFB_1) contents

The quantitative method ELISA (enzyme-linked immune sorbent assay) was used to determine the levels of AFs and AFB_1 in the samples of snail fleshes as well as their feed. The mycotoxins were extracted from the samples following the protocol defined by the manufacturer (Max-Signal® ELISA test kits, BIOO Scientific Corp., USA) for each type of matrix. In the experimental protocol, 2 g of snail flesh samples were taken from the 50 g of ground flesh previously prepared and mixed with 8 mL of 87.5% methanol (HPLC grade, Sigma, Germany). The mixture was vortexed for 10 min (Vortex Genius 3, IKA, Germany), centrifuged at 4000 g for 10 min (Centrifuge Rotofix 32 A, Germany) and the supernatant was collected. Regarding the feed sample, 5 g was taken and mixed with 25 mL of methanol 70%. The mixture was vortexed for 10 min, centrifuged (4000 g, 10 min), and the supernatant was collected. The collected supernatants were submitted to competitive direct ELISA. The 96-wells flat-bottomed plastic tissue plates were prepared following the manufacturer instruction and optical density (OD) were immediately read at 450 nm using an automated microplate reader (EL x 800, BIOTEK, Instruments Inc., Winooski, VT, USA). Standard solutions of AFs and AFB_1 0, 0.05, 0.25, 0.75, 2.5 and 10 ppm were used to plot the calibration curves ($r^2 = 0.99$) that were used for the calculation of AFs and AFB_1 contents the different samples. Samples with AFs and AFB_1 levels below 1 ppb which is the limit of detection specified by the kit's manufacturer were considered as containing AFs and AFB_1 at no detectable level.

2.7. Proximate composition of snail fleshes

The moisture of snail flesh samples was determined using the gravimetric method AOAC (1995). The ash content was assessed following the method AOAC (1990) while the protein content was estimated by the method of Devani et al. (1989), lipids by the method of Bourely (1982) and total sugars by the method of Dubois et al. (1956). Iron and zinc contents of the snail samples were determined using a Perkin-Elmer AAnalyst 400 atomic absorption spectrophotometer (AAS, Norwalk, USA), equipped with a graphite furnace and a deuterium lamp for background correction. Hollow cathode lamps (Perkin-Elmer) of Fe (λ = 248.33 nm, slit = 1.8/1.35 nm) and Zn (λ = 213.86 nm, slit = 2.7/1.8 nm) were used.

2.8. Statistical analyses

All experiments were repeated at least three times. The data obtained were expressed as mean ± standard deviation. Analysis of variance (ANOVA) and Tukey's test were used to compare means at $p < 0.05$. These analyses were performed using Minitab 16 software (Minitab Ltd., Coventry, UK). Principal component analysis was performed using XLSTAT 2018 (Addinsoft, Inc., New York, USA) to visualize the association between the microbial loads of the flesh samples, their chemical composition snail species and their AFB_1 and AFs contents.

3. Results

3.1. Morphometric characteristics of snail samples

The morphometric parameters of the three species of snails used in this study were measured and the results obtained are depicted in Table 1. The highest size (10.00 ± 0.01 cm) was recorded with the shell from *A. marginata*. It was significantly different ($p < 0.05$) of the shell sizes of the two other species. Although not significantly different ($p > 0.05$), the size of shells from *A. achatina* was higher than that of *A. fulica* (Table 1). Despite its low shell size compared to *A. marginata*, *A. achatina* showed a total weight of 86.56 ± 10.56 g which was not significantly different ($p > 0.05$) from that of *A. marginata* (80.98 ± 0.01 g). The less heavy snail species was *A. fulica* (49.13 ± 6.91 g). Similar observations were noticed with shell weights. They were 9.04 ± 3.39 g for *A. fulica*, 18.05 ± 1.00 g for *A. marginata* and 20.30 ± 6.37 g for *A. achatina*. However, regarding the flesh weight, a significant ($p < 0.05$) variation between the snail species was noticed (Table 1). The heaviest flesh was from *A. marginata* (33.55 ± 1.00 g). The fleshes from *A. achatina* and *A. fulica* showed weights of 28.13 ± 1.54 and 20.34 ± 2.88 g, respectively.

Taking into consideration the ratio of the flesh weight on the total weight of the three species of snails, *A. fulica* and *A. marginata* with a flesh ratio of 0.41 could be recommended for rearing compared to *A. achatina* for which a flesh ratio of 0.32 was obtained. However, the safety and nutritional quality of these fleshes should be considered as the main selection criteria.

3.2. Microbiological quality of snail flesh samples

As observed in Table 2, all snail flesh samples contained high levels of TAMF with loads ranging from 6.14 Log CFU/g (*A. fulica*) to 6.53 Log CFU/g (*A. achatina*). The TAMF count recorded with the highest contaminated sample (*A. achatina*) was not significantly ($p > 0.05$) different to that obtained with the sample (*A. marginata*). The total coliforms count of the flesh samples from the three snail species varies significantly ($p < 0.05$) from one sample to another and were 5.35 ± 0.04, 4.77 ± 0.07 and 5.16 ± 0.01 Log CFU/g for *A. achatina*, *A. fulica* and *A. marginata*, respectively. The same tendency was noticed with fecal coliforms as the flesh samples from *A. fulica* scored the least count (4.18 ± 0.07 Log CFU/g) while those from *A. achatina* and *A. marginata* showed loads that were not significantly ($p > 0.05$) different. The pathogens *E. coli* was found in all samples. Flesh samples from *A. achatina* were the most contaminated one with 4.55 ± 0.10 Log CFU/g. Although not significantly ($p > 0.05$) different, flesh samples from *A. fulica* and *A. marginata* showed loads of 3.57 ± 0.02 and 3.52 ± 0.66 Log CFU/g, respectively. *Salmonella* spp. and sulfite-reducing *Clostridium* were not found in any sample (Table 2). With regards to *Staphylococcus* spp., it was found in all flesh samples. However, a deep observation of Table 2 revealed that flesh samples from *A. achatina* scored a load that was not significantly different ($p > 0.05$) from that of *A. fulica*. The highest contamination in *Staphylococcus* spp. was observed with the flesh samples

Table 1. Mean values of the main morphometric parameters of the three species of snails used in this study.

Parameters	Snail species		
	A. achatina (n = 10)	*A. fulica* (n = 10)	*A. marginata* (n = 10)
Shell size (cm)	7.58 ± 0.42^a	7.28 ± 0.72^a	10.00 ± 0.01^b
Total weight (g)	86.56 ± 10.56^b	49.13 ± 6.91^a	80.98 ± 0.01^b
Shell weight (g)	20.30 ± 6.37^b	9.04 ± 3.39^a	18.05 ± 1.00^b
Flesh weight (g)	28.13 ± 1.54^b	20.34 ± 2.88^a	33.55 ± 1.00^c

N = number of samples; Values with different letters within the same raw are significantly different at $p < 0.05$.

Table 2. Microbial loads (Log CU/g) of the flesh samples from three different species of snails farmed in a locality (Mimboman) of the city of Yaoundé, Cameroon.

Germs	Snail species			Norms
	A. achatina (n = 10)	*A. fulica* (n = 10)	*A. marginata* (n = 10)	
TAMF	6.53 ± 0.10^{b}	6.14 ± 0.03^{a}	6.48 ± 0.11^{b}	5.47
Total coliforms	5.35 ± 0.04^{c}	4.77 ± 0.07^{a}	5.16 ± 0.01^{b}	3.00
Fecal coliforms	4.75 ± 0.15^{b}	4.18 ± 0.07^{a}	4.90 ± 0.02^{c}	1.00
E. coli	4.55 ± 0.10^{b}	3.57 ± 0.02^{a}	3.52 ± 0.66^{a}	0
SR-*Clostridium*	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.47
Staphylococcus spp.	2.66 ± 0.22^{a}	2.50 ± 0.20^{a}	3.44 ± 0.03^{b}	2.00
Yeasts and moulds	4.17 ± 0.07^{c}	3.50 ± 0.07^{a}	3.78 ± 0.04^{b}	/
Salmonella spp.	-	-	-	-

n = number of samples, TAMF = total aerobic mesophilic flora, SR-*Clostridium* = sulfite-reducing *Clostridium*. Values with different letters within the same raw are significantly different at $p < 0.05$.

from *A. marginata* (3.44 ± 0.03 Log CFU/g). Yeasts and moulds were present in all samples at loads that vary significantly with the snail species (Table 2). The most contaminated sample was from *A. achatina* (4.17 ± 0.07 Log CFU/g) while the least contaminated one was from *A. fulica* (3.50 ± 0.07 Log CFU/g).

3.3. Total aflatoxins and aflatoxin B_1 levels of flesh samples from the three species of snails

AFs and AFB_1 were detected in all samples analyzed in this study as showed in Table 3. The flesh samples from *A. fulica* were least contaminated with both AFs and AFB_1. They scored AFs and AFB_1 contents of 0.190 ± 0.001 and 0.095 ± 0.001 ppb, respectively (Table 3). These values were significantly ($p < 0.05$) different from those recorded with flesh samples from *A. achatina* and *A. marginata* for which respective values of 0.434 ± 0.151 and 0.403 ± 0.004 ppb were obtained for AFs, and respective values of 0.217 ± 0.075 and 0.201 ± 0.002 ppb for AFB_1. Food used for snails feeding was also contaminated with AFs and AFB_1 at levels of 0.114 ± 0.001 and 0.057 ± 0.001 ppb, respectively (Table 3).

3.4. Proximate composition of flesh samples from the three species of snails

The proximate composition of the flesh samples from the three species of snails analyzed in this study is shown in Table 4. The moisture of flesh from *A. fulica* (80.99 ± 0.79%) was significantly ($p < 0.05$) higher than that of the other species for which no significant variation was noticed. Protein contents of flesh from *A. achatina* (15.26 ± 0.54%) and *A. fulica* (14.83 ± 0.27%) were not significantly different, although the highest protein content was recorded with flesh from *A. achatina*. The lowest protein content was observed with flesh samples from *A. marginata* (12.48 ± 0.38%). A similar observation was noticed for sugar contents of flesh samples as the lowest content was noticed with flesh samples from *A. marginata* (2.37 ± 0.02%) and the highest content with those from *A. achatina* (3.52 ± 0.02%). No significant ($p > 0.05$) difference was noticed regarding the lipid contents of flesh samples from the three species of snails (Table 4). Surprisingly, the ash content of flesh samples from *A. marginata* (7.42 ± 0.02%) was significantly higher than that of the other species of snails. Flesh samples from *A. fulica* scored the lowest ash content (0.56 ± 0.01%).

The mineral profile of the flesh samples from the three species of snails was assessed with a focus on some targeted minerals of great importance for meat matrix such as iron and zinc. The highest iron content was noticed with sample from *A. achatina* (7.80 mg/100 g of flesh). Samples from *A. fulica* and *A. marginata* presented iron contents of 5.60 and 4.45 mg/100 g of flesh, respectively (Table 4). Regarding zinc, the highest amount was detected in flesh samples from *A. marginata* (0.48 mg/100 g of flesh). Samples *A. fulica* scored a zinc content of 0.31 mg/100 g of flesh while those from *A. achatina* were very poor in zinc with a content of 0.07 mg/100 g of flesh (Table 4).

Table 3. Total aflatoxins (AFs) and aflatoxin B_1 (AFB_1) contents (ppb) of feed and flesh samples from three species of snails farmed in a locality (Mimboman) of the city of Yaoundé, Cameroon.

Samples		Aflatoxin B_1	Total aflatoxins
Snail flesh	*A. achatina* (n = 10)	0.217 ± 0.075^{b}	0.434 ± 0.151^{b}
	A. fulica (n = 10)	0.095 ± 0.001^{a}	0.190 ± 0.001^{a}
	A. marginata (n = 10)	0.201 ± 0.002^{b}	0.403 ± 0.004^{b}
Feed (n = 1)		0.057 ± 0.001	0.114 ± 0.001
Norms			

n = number of samples. Values with different letters within the same column are significantly different at $p < 0.05$.

3.5. Principal component analysis

Association between the microbial loads of the flesh from the different species of snails, their AFs and AFB_1 contents, and their chemical composition was visualized through principal component analysis. The factors selected for that principal component analysis were the microbial loads, the AFs and AFB_1 contents, and the chemical composition (proteins, sugars, lipids, ash, iron, zinc) of the three species of snails. Figure 3 shows the distribution of these factors on the axis system F1 × F2. As observed in Figure 3, four groups were formed depending on the relationship between factors. The first group contained ash, zinc, *Staphylococcus* spp. and the snail specie *A. marginata*. This result highlights the close relationship between the flesh from *A. marginata* and minerals. Thus, suggesting *A. marginata* as a good source of minerals. The second group that is opposed to the first one is composed of *E. coli*, proteins, lipids, sugars, iron and the snail specie *A. achatina* (Figure 3). This observation suggests that *A. achatina* is the most nutritious meat compared to other snail species despite its high contamination with foodborne pathogens such as *E. coli*. The third group is made of AFs, AFB_1, TAMF, total coliforms, yeasts, moulds and fecal coliforms. Opposite to that third group appeared the four one which contained only the snail specie *A. fulica*. This observation demonstrates that the flesh from the snail species *A. fulica* was the least contaminated sample with both mycotoxins and pathogens.

4. Discussion

The total aerobic mesophilic flora is generally used as an indicator to assess the quality, the shelf life and sometimes the post-harvest contamination of foods (Nyoagbe et al., 2016; Mouafo et al., 2020). In this study, the flesh from the three species of snails analyzed were all highly contaminated with TAMF counts ranging from 6.14 ± 0.03 to 6.53 ± 0.10 Log CFU/g. High TAMF counts in meat from snails of different genera were also reported in the literature. Temelli et al. (2006) recorded

Table 4. Proximate composition of the flesh samples from three species of snails farmed in a locality (Mimboman) of the city of Yaoundé, Cameroon.

Parameters	*A. achatina* (n = 10)	*A. fulica* (n = 10)	*A. marginata* (n = 10)
Moisture (g/100 g of flesh)	76.87 ± 0.32^{a}	80.99 ± 0.79^{b}	76.46 ± 1.09^{a}
Dry matter (g/100 g of flesh)	23.13 ± 0.33^{b}	19.01 ± 0.33^{a}	23.54 ± 1.10^{b}
Proteins (g/100 g of flesh)	15.26 ± 0.54^{b}	14.83 ± 0.27^{b}	12.48 ± 0.38^{a}
Total sugars (g/100 g of flesh)	3.52 ± 0.02^{c}	2.80 ± 0.02^{b}	2.37 ± 0.02^{a}
Lipids (g/100 g of flesh)	1.60 ± 0.28^{a}	1.22 ± 0.21^{a}	1.20 ± 0.73^{a}
Ash (g/100 g of flesh)	2.75 ± 0.14^{b}	0.56 ± 0.01^{a}	7.42 ± 0.02^{c}
Iron (mg/100 g of flesh)	7.80 ± 0.56^{c}	5.60 ± 0.27^{b}	4.45 ± 0.14^{a}
Zinc (mg/100 g of flesh)	0.07 ± 0.01^{a}	0.31 ± 0.03^{b}	0.48 ± 0.01^{c}

n = number of samples. Values with different letters within the same raw are significantly different at $p < 0.05$.

a TAMF count of 6.85 Log CFU/g with meat from *Helix aspersa*. Adegoke et al. (2010) obtained 8.16 Log CFU/g of total bacteria counts in meat from *A. fulica*. With the meat from *A. marginata*, Ebenso et al. (2012) noticed total bacteria counts of 7.39–8.19 Log CFU/g. Nyoagbe et al. (2016) found TAMF counts of 7.87 and 7.01 Log CFU/g with meat from *A. achatina* and *A. marginata*, respectively. This contamination could be attributed to the rearing conditions. In fact, the snails were reared in pens made with materials that allow contact with air, dust and insects. These latter might harbor microorganisms and contaminate snails. Besides the quality of feed, water used by the rearers could also represent a source of contamination. Danilova and Danilova (2019) incriminated in their studies, the pens where snails are kept for rearing that are exposed to dust, the cleaning process and the closest of the rearing pens from the roadway as the leading causes of snail contamination by microorganisms. All snail samples independently of the species showed TAMF counts higher than the recommended level which is 5.7 Log CFU/g (EC, 2005). Based on that, we cannot conclude that these samples are not suitable for human consumption because they will be submitted to several treatments including heating before being consumed. It will therefore appear interesting to assess the effect of the different treatments applied on the flesh before its consumption on the microbiological quality of this later.

Besides the TAMF that only indicates the general contamination of snail fleshes, several groups of microorganisms might be present and render the product unsuitable for human consumption as they are often associated to potential health risks. Total and fecal coliforms' counts as well as *E. coli* counts were all higher than the threshold values recommended by the norms (EC, 2005). That observation could be explained by the fecal contamination of the snails' habitat. Indeed, during snail rearing, the feces generated as well as dead snails are not always removed as required. In these conditions, the decomposed snails associated to the feces constitute a favorable environment for the proliferation of pathogens (Ekundayo and Fagade, 2005; Nyoagbe et al., 2016). Hence, snails that will be directly in contact with these latter will get contaminated. Several studies have also demonstrated the high contamination of snails' feces in pathogens such as coliforms and *E. coli* (Efuntoye et al., 2011; Cardoso et al., 2012). As consequence, the *E. coli* and coliforms counts of snails licking the slime of infected feces could be high (Nyoagbe et al., 2016). Another explanation could be the poor microbiological quality of feed and water used for snail rearing as highlighted by Nyoagbe et al. (2016). The flesh samples from *A. fulica* scored the lowest coliforms and *E. coli* counts. A similar observation was made by Barimah (2013). The author found that *A. achatina* contained more Enterobacteriaceae counts than *A. fulica*. This difference could be attributed to the natural peculiar habitat of snails which varies from one species to another. Indeed, in this study, all the snail species were reared in the same place with identical climatic conditions. Hence, the growth rate, as well as the feed

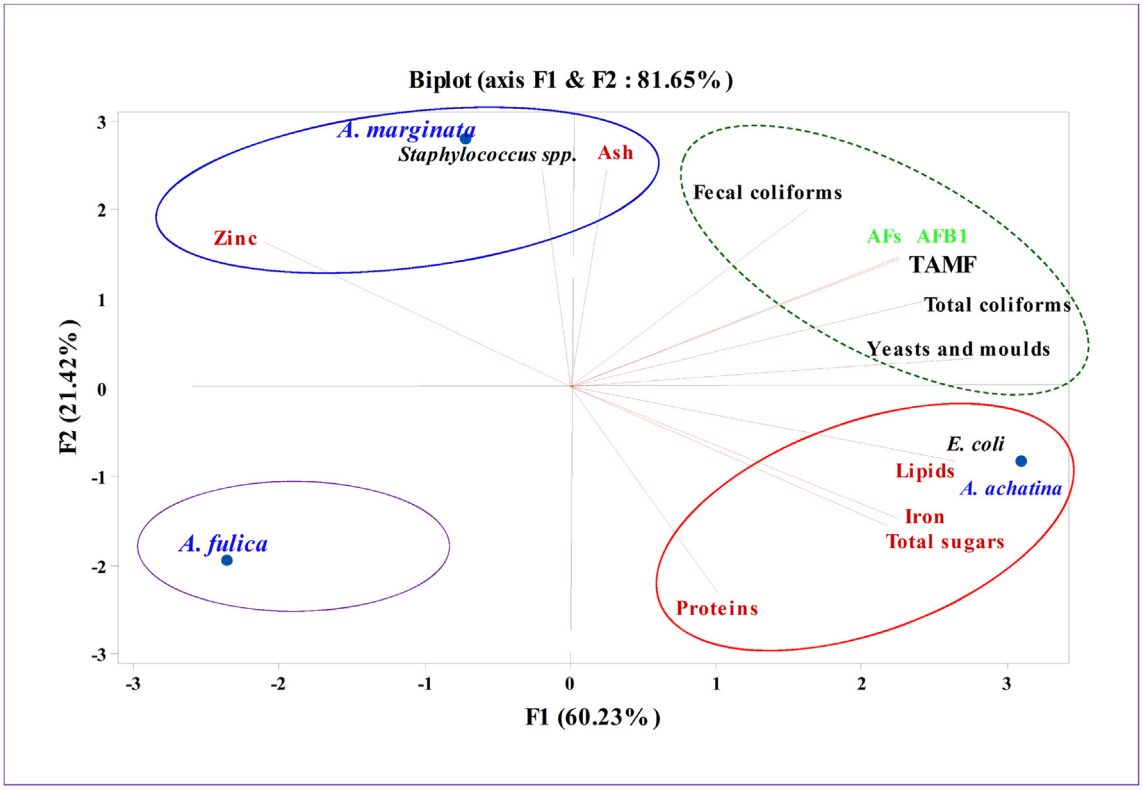

Figure 3. Distribution of the microbial loads, the total aflatoxins and aflatoxin B_1 contents of fleshes from the different species of snails and their chemical composition on F1 × F2 axis. TAMF = total aerobic mesophilic flora.

consumption (a potential source of microorganisms) that is associated to these conditions, might be different leading to contamination at various levels. The total coliforms count obtained in this study (4.77 ± 0.07 to 5.35 ± 0.04 Log CFU/g) were lower than that found by Nyoagbe et al. (2016). Authors reported loads of 7.47 and 6.75 Log CFU/g with meat from *A. achatina* and *A. marginata*, respectively. Adegoke et al. (2010) also noticed a total coliforms count of 7.30 Log CFU/g with the meat from *A. fulica*. However, they were higher than the total coliforms (2.77 Log CFU/g) and *E. coli* (2.56 Log CFU/g) counts of meat from *Helix aspersa* (Temelli et al., 2006).

It was very surprising to notice that notwithstanding the rearing conditions of snails, *Salmonella* and sulfite-reducing *Clostridium* were not detected in the flesh samples analyzed in this study.

Pathogens associated with food handling such as *Staphylococcus* spp. were also detected in the different flesh samples at loads ranging from 2.50 ± 0.20 to 3.44 ± 0.03 Log CFU/g. The frequent handling of snails by rearers as observed in the site could explain their contamination with *Staphylococcus* spp. Studies conducted by Adagbada et al. (2011) and Bukola et al. (2011) also indicated the presence of *Staphylococcus aureus* in snail meat. Temelli et al. (2006) found 3.96 Log CFU/g of *Staphylococcus* spp. in snail meat. The flesh from *A. fulica* was the less contaminated sample while that from *A. marginata* was the most contaminated one (Table 2). The observation made in this study was different from those reported by Nyoagbe et al. (2016). The authors noticed that meat from *A. achatina* contained more *Staphylococcus* spp. (4.96 Log CFU/g) than that from *A. marginata* (4.51 Log CFU/g). This difference could be ascribed to the feeding practices applied by the rearers. A similar conclusion was stated by Barimah (2013) during its investigation on the snails reared in Ghana. Raw fleshes from the different snails were all contaminated at loads higher than the norm which is 2 Log CFU/g (EC, 2005). Even though these bacteria are heat sensitive and can easily be eliminated during the cooking process, their ability to produce a heat stable enterotoxin (Brooks et al., 2004) in the snail flesh is worrisome as its consumption can lead to foodborne intoxination (Diasso, 2018).

Microorganisms with high spoilage activity such as yeasts and moulds were present in the different flesh samples analyzed in this study. The flesh from *A. achatina* was the most contaminated and the one from *A. fulica*, the least contaminated. The contact of snails with soil and dust present in their pens might be the source of contamination with yeasts and moulds. Barimah (2013) and Nyoagbe et al. (2016) also highlighted soil (host of several microorganisms) as the source of the microorganisms present in snails. In a study conducted by Bukola et al. (2011), several species of moulds such as *Aspergillus niger*, *Fusarium oxysporum* and *Cryptococcus* spp. were identified in snail meat. Loads of yeasts and moulds obtained in this study (3.50 ± 0.07 to 4.17 ± 0.07 Log CFU/g) were lower than that reported by Temelli et al. (2006). The authors noticed 5.63 ± 0.35 Log CFU/g of yeasts and moulds in meat from *Helix aspersa*. This difference could be attributed to the species of snail. In fact, the feeding habit of snails varies from one species to another. However, the spore-forming ability of moulds suggests that they could be present in flesh even after cooking and their consumption might lead to diseases. In addition to this, moulds can also produce in snail fleshes mycotoxins such as aflatoxins that are heat stable and the leading causes of mutagenic, neurotoxic, hepatotoxic, teratogenic, immunosuppressive, nephrotoxic and carcinogenic effects on humans (IARC, 2002; Zinedine and Mañes, 2009). Hence, its consumption might be a potential source of health risks for humans.

Giving that high load of moulds were found in the flesh samples, and knowing the mycotoxins producing-ability of this latter, it has appeared important to assess the presence of some mycotoxins such AFs and AFB_1 in these snails' meat. The results showed that these toxins were present in all flesh samples at ranges varying from 0.095 ± 0.001 to 0.217 ± 0.075 ppb for AFB_1 and 0.190 ± 0.001 to 0.434 ± 0.151 ppb for AFs. Their presence in flesh samples from locally farmed snails could originate from the feed that they consumed. The presence of 0.057 ± 0.001 ppb of AFB_1 and 0.114 ± 0.001 ppb of AFs in feed used for snail rearing as obtained in this study confirmed that hypothesis. In fact, during feed consumption, snails could accumulate AFs and AFB_1 in their tissue and the concentrations of these mycotoxins will increase as time passes (CAST, 2003; Shephard, 2008). This observation suggests the effects of snail age on the AFs and AFB_1 contents of their flesh. Besides, the presence of other major mycotoxins that can be found in snail feed such as ochratoxin A, fumonisins, deoxynivalenol, zearalenone and patulin should be assessed in the snail fleshes.

The levels of AFs and AFB_1 were significantly ($p < 0.05$) low in flesh samples from *A. fulica* while the ones from the two other species were not significantly different ($p > 0.05$) although *A. achatina* scored the highest values. The fact that the amount of feed ingested and the metabolic activities varies from one snail species to another could explain the difference observed. The maximum values of AFs and AFB_1 in food intended for human consumption are 10 and 5 ppb, respectively (FAO/WHO, 2017). Knowing the bioaccumulation ability of these mycotoxins (Shephard, 2008; Nguegwouo et al., 2019), their presence in snail fleshes even at concentrations below the maximum threshold values recommended by the FAO/WHO should attract particular attention.

An important way to valorize the snails reared locally passes through the evaluation of their nutritional values. The nutrient content of food is generally assessed via its proximate composition. In the present study, the proximate composition of flesh from three species of snails was determined. The fleshes of the different snails were all good sources of proteins (12.48 ± 0.38 to 15.26 ± 0.54%). The protein contents of these locally reared snails were comparable to that of conventional food animals such as beef, chicken, pork, and fish (Malik et al., 2011). This observation reveals that the sustainable production of snails should be encouraged as sometimes, mostly in the dry season, their price increase as highlighted by Ogogo et al. (2011). In fact, the production of snails does not require high capital investment and is affordable for the low-income populations who predominate in developing countries. With regards to the snail species, the highest protein content (15.26 ± 0.54%) was obtained with flesh from *A. achatina*. The protein content of flesh from *A. achatina* obtained in this study (15.26 ± 0.54%) was higher than that reported by Marcel et al. (2020) with the flesh of *A. achatina* reared in Ivory Coast (12.74%). The flesh from *A. fulica* scored a protein content (14.83 ± 0.27%) comparable to 14.48% reported by Fagbuaro (2015). However, the one from *A. marginata* (12.48 ± 0.38%) was lower than that recorded by Solomon et al. (2020) with the flesh of snail from the same species (17.22%). This variation could be attributed to the rearing conditions and the nature and type of feed used by farmers.

Independently of snail species, the lipid contents of fleshes although not significantly different (p˃0.05) were low (1.20 ± 0.73 to 1.60 ± 0.28%). This observation is similar to several reports in the literature (Ejidike and Oyekunle, 2019; Marcel et al., 2020; Solomon et al., 2020) and suggests the suitability of that food for human consumption particularly in this context where the prevalence of non-communicable diseases associated to lipids such as cardiometabolic diseases, hypertension and obesity are very high and increases as time passes.

Ash generally refers to the mineral content of the food. The ash content of the flesh from the three species of snails analyzed in this study varies significantly ($p < 0.05$) from one species to another. The flesh from *A. fulica* scored a very low ash content (0.56 ± 0.01%) while the one from *A. marginata* scored the highest content (7.42 ± 0.02%). A low ash content (0.81%) was also observed by Marcel et al. (2020) with flesh from *A. achatina*. This difference could be explained by the minerals' intake from soil and from food which varies with the snail species. In a study conducted by Fagbuaro et al. (2006), it was also noticed that the flesh from *A. marginata* contained more ash compared to other snail species.

Iron is among the key minerals that play important roles in human well-being such hemoglobin formation, enzymatic systems and neural development. Its deficiency might lead to severe adverse health effects including death (Meherunnahar et al., 2018). In this study, the highest iron content (7.80 mg/100 g) was recorded with flesh from *A. achatina*.

This result was different to those of Fagbuaro et al. (2006) highlighting that amongst snails' species, *A. marginata* contained more iron. The iron contents of snails analyzed in this study independently of the species were higher than the 3.5 mg/100 g recommended by the USDA (2006), and iron 1.42 mg/100 g reported by Marcel et al. (2020). However, it was lower than 9.53 mg/100 g recorded by Kehinde et al. (2020) and Solomon et al. (2020) with the flesh from *A. marginata*. This difference could be ascribed to the abundance of iron in the soil of the rearing site and the feed consumed by snails. According to the United States Department of Agriculture (USDA, 2018), the required daily intake (RDI) of iron in food intended to be consumed by humans is 0.27 mg/day for infants younger than 6 months, 8 mg/day for children between 9–13 years per day is and between 8–18 mg/day for others. The values of iron contents of snails analyzed in this study (4.45–7.80 mg/100 g) suggest that the consumption of 200 g per day of these fleshes can adequately meet the RDI. For consumption of less than 200 g per day, snail fleshes should be associated with other dietary sources of iron. The presence of iron in snail fleshes suggests that it could be suitable for women at child-bearing age, pregnant and for nursing women. However, they should overpass some superstitious beliefs stating that snail consumers might become sluggish or slow.

Zinc is an essential mineral for all living cells. It is important for the functioning of several enzymes generally called known as metalloenzymes (Soetan et al., 2010) and for the metabolism of nucleic acids and proteins as well as their cell division, differentiation and development (Parul and West, 1998). Zinc also deserves antiviral activity and was reported to be active against SARS-Cov-2 (Wu et al., 2020; Zhou et al., 2020). In the context of the COVID-19 pandemic, the presence of zinc in food might confer potential health benefits to that later. In this study, the zinc content of snail fleshes was assessed and the highest value of 0.48 mg/100 g was obtained with samples from *A. marginata*. The richness in minerals of snail from the species *A. marginata* compared to others was also reported by Fagbuaro et al. (2006). The zinc content obtained in this study was lower than those reported in the literature by the USDA (2006) which is 1 mg/100 g, by Oluwatosin (2019) with the flesh from *A. marginata* (1.03–1.13 mg/100 g) or by Fagbuaro (2015) with the flesh from *A. marginata* (1.88 mg/100 g). However, the zinc contents of the different flesh samples (0.07–0.48 mg/100 g) do not meet the RDI which is 2–8 g/day for infants and children and 11–13 g/day for adolescents and adults (USDA, 2018). Hence, snails reared in the quarter of Mimboman (city of Yaoundé, Cameroon) independently of the species are not suitable to cover the RDI of zinc. This result suggests that improvement should be made on the feed used to rear these animals and the quality of the soil should be monitored. In fact, in the rearing process of snails, soil and feed were reported as key factors to improve the nutritional quality and thus the mineral profile of snail fleshes (Oluwatosin, 2019).

From principal component analysis depicted in Figure 3, the snail species *A. marginata* was closely associated with ash and zinc. This could be ascribed to the high mineral intake ability of that snail species as suggested by Fagbuaro et al. (2006). This result suggests that the consumption of *A. marginata* meat might boost the immune system and protect the body against several diseases. However, specific attention should be taken during its cooking as its raw flesh contained high loads of *Staphylococcus* spp. The most nutritious snail meat was the one from the species *A. achatina*. It was positively associated with proteins, lipids, total sugars and iron (Figure 3). This observation demonstrates the suitability of that meat to fight against malnutrition for which the prevalence is high in several low-income countries. Hence, the sustainable production of *A. achatina* should be encouraged. However, the relationship observed between the flesh from species *A. achatina* and pathogens like *E. coli* suggests that its consumption might be associated to foodborne diseases. That contamination originated from the rearing environment as well as the feed and water ingested by the snail (Barimah, 2013; Nyoagbe et al., 2016) can be reduced through the application of good hygiene and rearing practices. Hence, a sensitization of these snail rearers might lead to an improvement in the quality of these nutritious meats. Although less nutritious than the other snail species, the flesh from *A. fulica* was the least contaminated with both mycotoxins and pathogens. Besides, the highest flesh yield (0.41) was also noticed with snails belonging to that species. In order to avoid health risks associated to the consumption of snails, the results of this study advise the choice of the snail species *A. fulica*. It also suggests that more studies should be performed on the feeding habit of that species in view of enhancing their nutritional properties.

5. Conclusions

This study revealed that the raw fleshes of the different species of snails traditionally reared in the city of Yaoundé were of poor microbiological quality. They contained several pathogens which rendered questionable the safety issue of these meat products. The fact that snails' meat is consumed either cooked or roasted might considerably reduce these contaminations and make the product suitable for human consumption. However, the presence of microorganisms with spores-forming and toxins-producing abilities which heat stable suggests that particular attention should be paid to these products in order to protect consumers' health. AFs and AFB_1 were detected in snail flesh at a level lower than that recommended by the FAO/WHO norms for food intended for human consumption. Notwithstanding this, the regulation on that specific meat product should be created and adopted by the government for a better valorization of these locally reared snails.

Declarations

Author contribution statement

Linda Manet: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Roger Moïse Mbanga Baleba: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Patrice Bonny: Performed the experiments; Wrote the paper.

Jean David Pool Likeng: Conceived and designed the experiments; Performed the experiments.

Hippolyte Tene Mouafo: Analyzed and interpreted the data; Wrote the paper.

Gabriel Nama Medoua: Contributed to reagents, materials, analysis tools.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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