Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 15(1): 34-40, February 2021

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Microbiological analysis of the *Cirina forda* (Lepidoptera: Saturniidae) commercialized in North Togo

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ACKNOWLEDGMENTS

The authors thank the International Foundation for Science (IFS) for the funding that enabled this work to be carried out.

Received: 05-10-2020	Accepted: 20-02-2021	Published: 28-02-2021

ABSTRACT

Edible insects are presented as a source of protein, fat and micronutrients. Consequently, they are an attractive growing environment for microorganisms. Although, professionals in this sector in Sub-Saharan Africa use traditional harvesting, processing and marketing techniques, there is little scientific data on the microorganisms that infest edible insects**.** The aim of this study was to identify the microorganisms present in the *Cirina forda* supply chain, the most commercially marketed insect species in Togo. A total of 300 samples of fresh, processed and commercial *C. forda* caterpillars were collected and analyzed using standard microbiological microorganism identification techniques. The caterpillar was 90% contaminated with *Staphylococcus spp*, 60% by *Escherichia coli*, 40% by *Enterobacter spp*, 40% by *Aspergillus niger*, 30% by *Klebsiella pneumoniae* and 10% by *Mucor spp*, *Klebsiella oxytoca*, *Proteus spp*, *Serratia spp* and *Aspergillus flavus*. Pathogenic microorganisms are found in all samples at all stages of the supply chain, so caterpillars handled using traditional methods are detrimental to the health of the consumer. The presence of germs indicative of contamination of the caterpillar analyzed exposes consumers to the risk of food poisoning.

Keywords: *Cirina forda*, marketing, microorganisms, Togo.

DOI : https://dx.doi.org/10.4314/ijbcs.v15i1.4

INTRODUCTION

Human consumption of insects is a traditional practice in some parts of the world, including Asia, Africa and Latin America. Around 2,086 species of insects are consumed worldwide, across 130 countries and by 3,071 ethnic groups (Ramos-Elorduy, 2009). The United Nations Food and Agriculture Organization (FAO) initiated work in 2008 to promote the consumption of insects as a source of protein, fat and micronutrients (van Huis et al., 2014). According to the FAO, insects are the traditional meals of at least two and a half billion people (van Huis et al., 2014). The most widely eaten insects are beetles (Coleoptera, 28%), bees, wasps and ants (Hymenoptera, 21%), locusts and crickets (Orthoptera, 16%), caterpillars (Lepidoptera, 15%) (Johnson, 2010). Van Huis (2003) notes that in sub-Saharan Africa, caterpillars (Lepidoptera, 30%) are the most widely consumed insects. In addition, specific work has done out on edible caterpillars in Africa. Saturniidae, Notodontidae and Sphingidae are the three major families of Lepidoptera whose caterpillars are most consumed (Malaisse et al., 2003). Saturniidae larvae, especially larger sizes, are very popular in many Africans countries. At the time of their appearance, they are harvested massively. They are highly valued and exported, sub-regionally and to diasporas in Europe (Tabuna, 2000). Among the edible Saturniidae species, the caterpillar *Cirina forda* is the most accepted food item in Africa : Nigeria, Zambia, Zimbabwe, South Africa, the Central African Republic, the Democratic Republic of Congo, Togo (Yabuda et al., 2019). In Nigeria and Togo in particular, this caterpillar has become the most commercialized insect species (Agbidye et al., 2009; Badanaro et al., 2015). In Togo, the Savannah Region markets of this caterpillar. Although this resource is the subject of a flourishing trade in this region, little research has been done to promote it. Badanaro et al. (2014) have shown that the *C. forda* caterpillar consumed in the Savannah Region of Togo is a good source of protein, fat, fiber and minerals. However, they are an attractive growing environment for microorganisms. Like other caterpillar species consumed in Togo, *C. forda* is collected from the ground, while not always respecting hygienic and sanitary conditions. Moreover, it must be recognized that the conservation of caterpillars in Togo (a hot country) is often difficult because of the highly perishable nature of the commodity, the lack of adequate conservation infrastructure and especially the climatic and environmental conditions promoting its rapid degradation. This situation has led to professionals in this sector using traditional techniques for processing and conserving caterpillars. The marketing methods of this caterpillar are also traditional. A large amount of caterpillars is produced each year in Togo. Its production presents very interesting economic and commercial interests, hence the need to promote the *C. forda* sector in Togo. With a view to developing the *C. forda* sector, caterpillars for human consumption sold to consumers must meet the same safety and hygiene standards as all other foods. For this reason, this study aimed at determining the microorganisms carried by the *C. forda* caterpillar throughout its supply chain (from harvesting to the consumer) in order to identify critical factors that may influence the quality of the caterpillar as food item.

MATERIALS AND METHODS

Site and study period

Samples were collected in the village of Malagou (Nano) and at the market in the city of Dapaong in the Savannah Region of Togo during August 2018. The collection and reception equipment consists of 500 ml sterile bottle and a cooler with refrigeration elements. Sterile gloves were used to collect the caterpillars. Fresh and processed caterpillars were collected from shea plants, pickers who are at the same time processors, wholesalers, semi wholesalers, retailers and random consumers. From each source (step), 30 samples of caterpillars were collected. The microbiological analyses were carried out at

the Laboratory of Biomedical Sciences, Food and Environmental Health (LaSBASE) of the Advanced School of Biological and Food Technology (ESTBA) at the University of Lomé in the same period according to Bawa et al. (2017).

Bacterial culture and identification

The caterpillars in each group, collected at random, were introduced in a tube containing 10 ml of nutrient broth for the culture of bacteria. The inoculated broth was stirred using a Vortex agitator for 3 minutes to suspend the germs from the caterpillar exoskeleton. The tubes were then incubated at 37 °C for 3 hours. The culture was then performed on the following agar media: Chapman, Blood Agar, Mac Conkey and Eosin Methylene Blue Agar (EMB). Three petri dishes from each medium were seeded and incubated at 37 °C for 24 hours. The blood agar petri dishes were incubated in the presence of 5% CO_2. Each type of isolated colonies was then subcultured on the same media in order to obtain pure cultures before the identification tests. The identification of bacteria was based on the study of their biochemical characteristics. After controlling Gram coloration, the search for catalase and coagulase was performed on Gram-positive cocci. From the EMB and Mac Conkey media, a classic enterobacteriaceae identification gallery composed of Simmons' Citrate, Kligler-Hajna, Mannitol-mobility and Indole Urea media was seeded on each isolated colony to identify Gram-negative bacilli. All the galleries were incubated at 37 °C for 18 to 24 hours. The galleries were read after incubation and then the bacillus were identified using the dichotomous enterobacteriaceae identification table. In addition to these tests, the oxidase test was conducted on Gram-negative bacillus that did not ferment glucose.

Fungi culture and identification

Fungi were isolated from caterpillar lots collected at each stage by direct contact on Potato Dextrose Agar (PDA) and Sabouraud chloramphenicol. The fungi isolation technique is as follows: 2 caterpillars were randomly selected and placed on the surface of the agar. Three petri dishes were seeded in groups and incubated at 25 °C for 7 days. The plates were observed after 72 hours and then after 7 days. When the culture is positive, subculture was done on the PDA agar for pure culture before identification. The identification was based on macroscopic and microscopic examinations. The macroscopic examination involved identifying the characteristics visible to the naked eye: the appearance of the mycelium, its colour on the front and back. Microscopic examination of the fungi was carried out by using lactophenol blue cotton. The identification was done by referring to Barnett. (1967) and Botton et al. (1990).

Data processing

All tests were carried out in triplicate. The data was processed using the Excel 2013 spreadsheet©.

RESULTS

The isolated germs at different steps of the *C. forda* supply chain are presented in Table 1. The results of the microbiological analysis of the *C. forda* samples show that the caterpillars were contaminated. *Staphylococcus spp* (80%) was found in the majority of samples analyzed, followed by *Escherichia coli* (60%). *Aspergillus niger* (40%), *Klebsiella pneumoniae* (30%), *Enterobacter spp* (20%), *Enterobacter aerogenes* (20%), *Klebsiella oxytoca* (10%), *Proteus spp* (10%), *Serratia spp* (10%), *Mucor spp* (10%), *Staphylococcus aureus* (10%) and *Aspergillus flavus* (10%) were also found in the samples (Figure 1). Table 2 shows the contamination rates of *C. forda* at different stages of the supply chain. The highest contamination rates are recorded for harvest stages and marketing ranged from 50 to 100%. Contamination rates in the processing stages are relatively lower (less than 50%).

Table 1: Isolated Germs at different steps of the *C. forda* supply chain.

Steps	Isolated germs
1. Caterpillars sampled at the site	*Staphylococcus spp, Escherichia coli, Enterobacter aerogenes, Aspergillus niger*
2. Fresh caterpillars sampled from Pickers	*Staphylococcus spp, Klebsiella oxytoca, Enterobacter spp, Aspergillus niger*
3. Caterpillars sampled after boiling by pickers	*Staphylococcus spp, Escherichia coli, Klebsiella pneumoniae*
4. Caterpillars sampled after boiling and drying	*Staphylococcus spp*
5. Caterpillars sampled after roasting	*Staphylococcus spp, Klebsiella pneumoniae*
6. Caterpillars sampled after roasting and drying	*Staphylococcus spp, Escherichia coli*
7. Caterpillars purchased from producers	*Staphylococcus aureus, Escherichia coli, Enterobacter spp, Pseudomonas spp, Aspergillus niger, Mucor spp*
8. Caterpillars purchased from wholesalers	*Staphylococcus spp, Proteus spp, Escherichia coli*
9. Caterpillars purchased from semi-wholesalers	*Enterobacter aerogenes, Escherichia coli, Pseudomonas spp*
10. Caterpillars purchased from retailers	*Staphylococcus spp, Klebsiella pneumoniae, Serratia spp, Aspergillus niger, Aspergillus flavus*

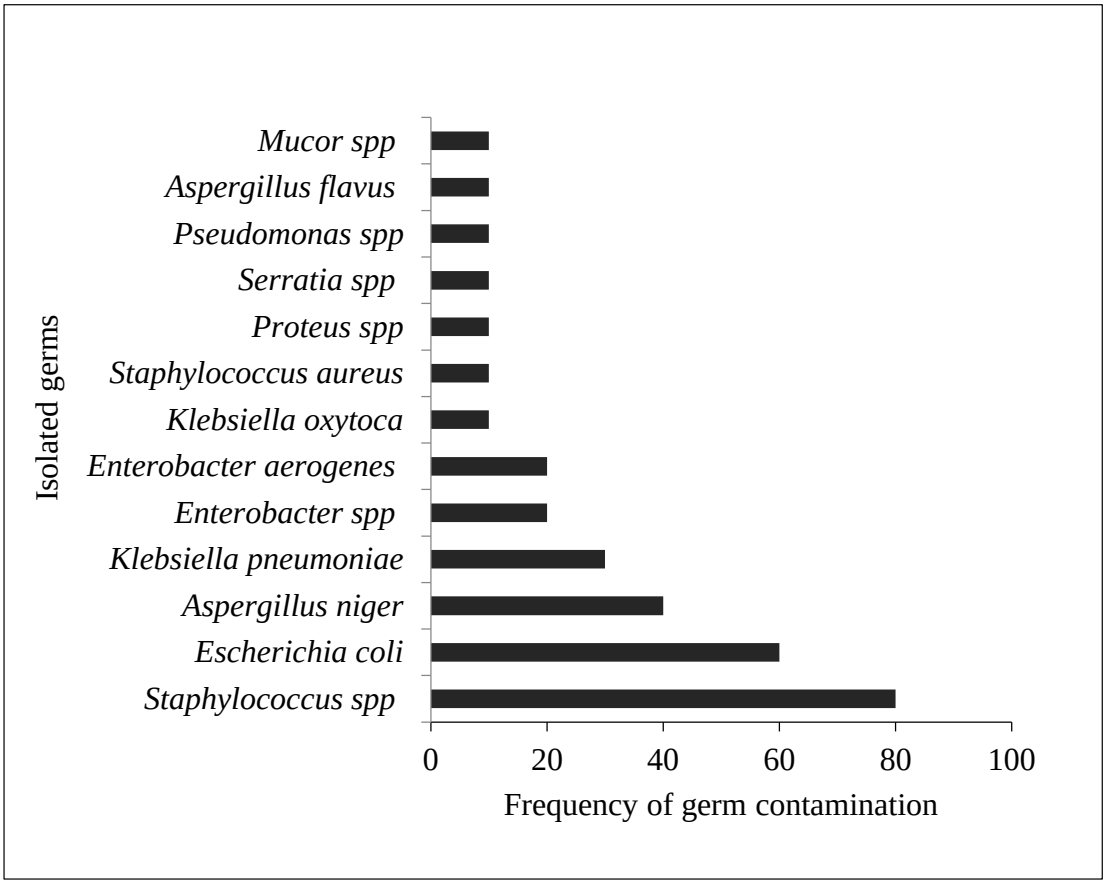

Figure 1: Frequency of contamination of caterpillars by isolated germs.

Table 2: Contamination rate (%) at the different steps of the *C. forda* supply chain (n = 30 per step)

Steps	1	2	3	4	5	6	7	8	9	10
Number of positive samples	22	22	15	10	10	14	30	15	14	17
Contamination rate (%)	73.3	73.3	50	33.3	33.3	46.6	100	50	46.6	56.6

DISCUSSION

Like any animal, insects are not immune from diseases caused by bacteria, viruses and fungi (Dillon and Charnley, 2002; Vega and Kaya, 2012). However, insect pathogenic microorganisms sometimes belong to taxonomic groups different from those of humans. They have an entirely different life cycle from human microorganisms and would pose no danger to humans (van Huis, 2013). In the genus *Bacillus*, the pathogen of the insects *Bacillus thuringiensis* and that of the vertebrates *Bacillus anthracis* appear to have totally different life cycles. *B. thuringiensis* is a pathogen in insects but is harmless in humans (van Huis, 2013). Indeed, because insects are taxonomic much further from humans than livestock; they may be thought to be at low risk of transmission of zoonoses such as avian influenza H1N1 and mad cow disease (bovine spongiform encephalopathy). On the other hand, pathogenic microorganisms can cause disease in humans or produce toxins that can proliferate on insects if hygiene measures are insufficient during the supply, processing and marketing processes (Johnson, 2010). According to this study, the microorganisms are carried by the caterpillar *C. forda* throughout the chain (from the site to the consumer). The caterpillars are more infested for at the collection and marketing stages than during processing. Reducing the microbial load during caterpillar processing demonstrates that the processing methods used are effective in reducing initial contamination. The increase in microbial load during the marketing of the caterpillar after transformation would be due to the lack of hygiene in the handling of the caterpillar. Factors contributing to the increased potential risks to the caterpillar along the marketing chain appear to be inadequate hygiene at all stages of the commercial chain, such as inadequate or unsuitable storage conditions and exposure to air. The results of this study corroborate those of other studies on edible insects in Africa. In Nigeria, pathogenic bacteria including *S. aureus, P. aeruginosa* and *B. cereus* were isolated from *Oryctes monoceros* (Coleoptera: Scarabaeidae) that was partially dried, fried and sold in markets (Banjo et al., 2006). The *C. forda* samples studied are contaminated in the collection environment by several pathogenic microorganisms including *Staphylococcus spp, E. coli, E. aerogenes* and *A. niger*. Therefore, the products upstream of the sector are not of good quality. The infestation of caterpillars could come from the collection environment as the caterpillars are collected from the wild by villagers whose household waste is released in the environment and who do not have latrines. However, our study did not focus on the remediation of the caterpillar collection medium. Contamination of caterpillars could also be caused by inadequate collection practices. Furthermore, this study shows that samples are contaminated in 90% of cases with *Staphylococcus* and 60% of cases with *E. coli*. Of the germs found, *Staphylococcus spp* is the most represented. Its presence is evidence of poor hygiene (Sandel and McKillip, 2004; De Buyser and Sutra, 2005). *S. aureus* found in the samples is a bacterium that causes common skin-mucous infections such as panaris, boils, and various abscesses (De Buyser and Sutra, 2005). *E. coli* is the species that comes second in terms of representation. As *E. coli* is a common presence in human and animal guts, it is sought as a germ that controls fecal contamination. The presence of fecal contamination-indicator germs in the samples analyzed exposes consumers to the risk of gastroenteritis (HCSP, 2015 ; Ouendo et al., 2015). *E. coli* infection can cause serious food borne illnesses such as abdominal cramps and diarrhea, which in some cases progress to bloody diarrhea (Ahoyo et al., 2010). There may also be fever and vomiting (EFSA, 2007).

Other pathogenic germs like *Enterobacter spp*, *K. pneumoniae*, *K. oxytoca* and *Proteus spp* found in the samples are responsible for urinary tract and other infections (Toudji et al., 2017 ; Yandai et al., 2019). The fungi detected in the samples of the caterpillars studied are *Aspergillus niger A. favus* and *Mucor spp*, species can cause fungal infections (Halewyn et al., 2002). As a result, caterpillars are contaminated with pathogens. Their consumption can lead to ingestion of pathogens that cause food borne illnesses.

Conclusion

The caterpillars in the *C. forda* sector in north Togo do not appear to be produced according to good collection, processing and, above all, marketing practices. Pathogenic microorganisms are found in all steps throughout the *C. forda* supply chain. The increasing spread of insect-based diet is accompanied by risks in terms of microbiological quality.

COMPETING INTERESTS

The authors have declared that there is no competing interest.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. FB, KA and KSA designed the project; BF carried out the sampling; BF and YH carried out the experiments; FB and YH analyzed the data; BF, YH and KAG wrote the manuscript; AK oversaw all activities; all authors agreed to the final version of the manuscript and its publication.

ACKNOWLEDGMENTS

The authors thank Dr Wilson-Bahun Tetevi for his help in producing the English version of this manuscript.

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