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Heavy metals and pathogenic bacteria detected in fermented cassava dough and *attiéké* sold in Ouagadougou, Burkina Faso

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ABSTRACT

Attiéké, a local processed cassava roots took place in population dietary habit. This widely consumed street vended a*ttiéké* is sometimes contaminated by chemical compounds and bacteria due to the growth soil and poor hygiene during processing. This study was carried out to assess the heavy metals and microbial contamination of fermented dough and *attiéké* sold in Ouagadougou, Burkina Faso. A total of 60 samples of fermented cassava dough and *attiéké* were collected and tested by flame atomic absorption spectrometry for contamination by cadmium, lead and aluminum. Contamination by *Escherichia coli*, coliforms, *Staphylococcus aureus* and *Salmonella* spp. was carried out by microbiological standard methods. Dough samples means contamination by lead, cadmium and aluminum levels were respectively of 0.738, 0.006 and 1.011 mg/kg and 0.393; 0.003 and 0.492 mg /kg for *attiéké*. *Staphylococcus aureus* was detected in all samples with an average load of $1.80x10^4$ $CFU.g^{-1}$ in the dough and $1.49x10^4$ $CFU.g^{-1}$ in the *attiéké*. Thermotolerant coliforms were detected in 15% of the dough samples with an average load of $0.015x10^2$ $CFU.g^{-1}$. *Salmonella* was not detected. Contamination of dough and *attiéké* indicated a need of awareness on cassava, good agricultural and good hygienic practices during cassava processing into *attiéké*.

Keywords: Microbiological quality, Chemical compounds, Cassava, Dough, Awareness.

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INTRODUCTION

In Burkina Faso, there are some varieties of cassava used in *attiéké* production with several nutritional properties. In the production of *attiéké* (Cassava: *Manihot esculenta* root ground and fermented), handling operations are commonly used in artisanal processing units (Diancoumba, 2008). However, food contamination due to improper handling and processing, is a major public health concern (Anukwuorji et al., 2020). Indeed, the lack of good hygiene practices of *attiéké* producers, the poor storage and import conditions of fermented cassava dough and the lack of an adequate processing unit are generally a source of contamination and can expose the consumers at risk of foodborne diseases. Furthermore, the sale of foodstuffs on public roads and in markets, commonly observed in West Africa, could be an important factor in the contamination of these foods (Barro et al., 2006). The production of *attiéké* is done in a traditional way in inappropriate hygienic conditions leading to the contamination of food by heavy metals or bacteria (Heidarieh et al., 2013).

In addition, the contamination of food and especially cassava derivatives by heavy metals from raw material or the processing equipment (Capo-Chichi et al., 2019) and also from the agricultural fertilizers and the anthropogenic activities (FAO, 2018). The microbiological contamination is mostly due to unhygienic practices observed on the sites where these foods are sold (Barro et al., 2006). Contamination occurs at all levels in food chain, from farm to fork. *Salmonella* spp., *Escherichia coli* producing shiga-toxins, *Staphylococcus aureus*, *Listeria monocytogenes* are major bacteria contaminated *attiéké* (De Buyser et al., 2001).

Consumption of contaminated foods can cause serious health problems for the consumers. Indeed, they expose it to poisoning or foodborne diseases. In Burkina Faso, despite many studies on street vended-foods, street vended *attiéké* is mainly associated to diarrhea diseases in towns. Thus, this study aimed at assessing the levels of heavy metals and the microbiological quality of fermented cassava dough and *attiéké* sold in Ouagadougou.

MATERIALS AND METHODS

Study areas and sampling

Samples were collected from September 2018 to February 2019 in eighteen (18) sites in Ouagadougou, Burkina Faso (Figure 1). Sites were chosen, taking into account the high relative human density (train station, markets, hospitals, etc.). A random sampling was done with 60 products. However, we took 20 samples of fermented cassava dough and 40 samples of *attiéké* on the unloading and storage sites of the railway station (n =15 dough and 12 *attiéké*), in *attiéké* processing units (n =12 *attiéké*), in markets (n= 5 dough and 5 *attiéké*), in the street and modern restaurants (n =11 *attiéké*). Samples were packed in a sterile bag of 500 g each, stored at +4°C and immediately conducted in the laboratory and then analyzed within two hours. Each sample was labeled with the selling area, the origin and type of *attiéké* and fermented dough.

Bacteria identification and counting

The preparation of samples and tenfold dilution for inoculation out agar plates (Global Roll Petri Dishes, 90 mm) were carried out using standard methods. For all numerations, 25 g of the samples were homogenized in a stomacher bag containing 225 mL of sterile buffered peptoned water (Liofilchem, Italy). *Staphylococcus aureus* was isolated and enumerated according to the method used by Capita et al. (2001). A volume of 0.1 mL of each dilution was surface plated on Baird-Parker agar containing egg yolk tellurite emulsion (Oxoid, Dardilly, France) and incubated at 37°C for 24 and 48 h. For thermotolerant coliforms, 0.1 mL of each dilution was streaked into Violet-Red Bile Lactose (VRBL) agar (Conda Pronadisa, Spain) and plates were incubated at 44°C for 24 h. As for *E. coli* one to two colonies of VRBL medium were inoculated on Eosin Methylene Blue (EMB) medium and incubation was carried out for 24 hours at 37°C. *Salmonella* spp.

have been investigated according to ISO 6579 (2002) and 0.1 mL of pre-enriched broth was homogenized in 10 mL of Rappaport-Vassiliadis Soja (RVS) broth and incubated respectively at 42°C for 18 to 24 h. For the selective isolation, Xylose Lysine Deoxycholate agar (Liofilchem, Italy) was used. Strains were purified on Mueller Hinton agar and confirmed on minimal gallery and API 20E. The unacceptable microbiological limits for which ready-to- eat *attiéké* samples were considered potentially hazardous in this study was based on standards for ready-to-eat foods by the Committee on the Elaboration of Microbiological Criteria in Foods (CEMCF) (2009). It's ≥10 CFU.g^{-1} for *E. coli*; ≥ 10^2 CFU.g^{-1} for *S. aureus* and the absence of *Salmonella* spp. in 25 g of food samples.

Analysis of heavy metal contamination

The samples contamination by lead, cadmium and aluminum were determined by flame atomic absorption spectrometry (VARIAN 240FS, Australia). Wet digestion of samples was performed using a mixture of acids HNO_3/HCl (3:1), according to the method of Demirel et al. (2008) with slight modifications. A volume of 5 mL of the acid mixture were added to 0.5 g of dried samples and heated at 150°C for 2 h 30 min. An atomic absorption spectrometer VARIAN 240FS (Mulgrave, Australia), equipped with single and multi-element hollow cathode lamps was used for elementary analysis and under the conditions recommended by the manufacturer.

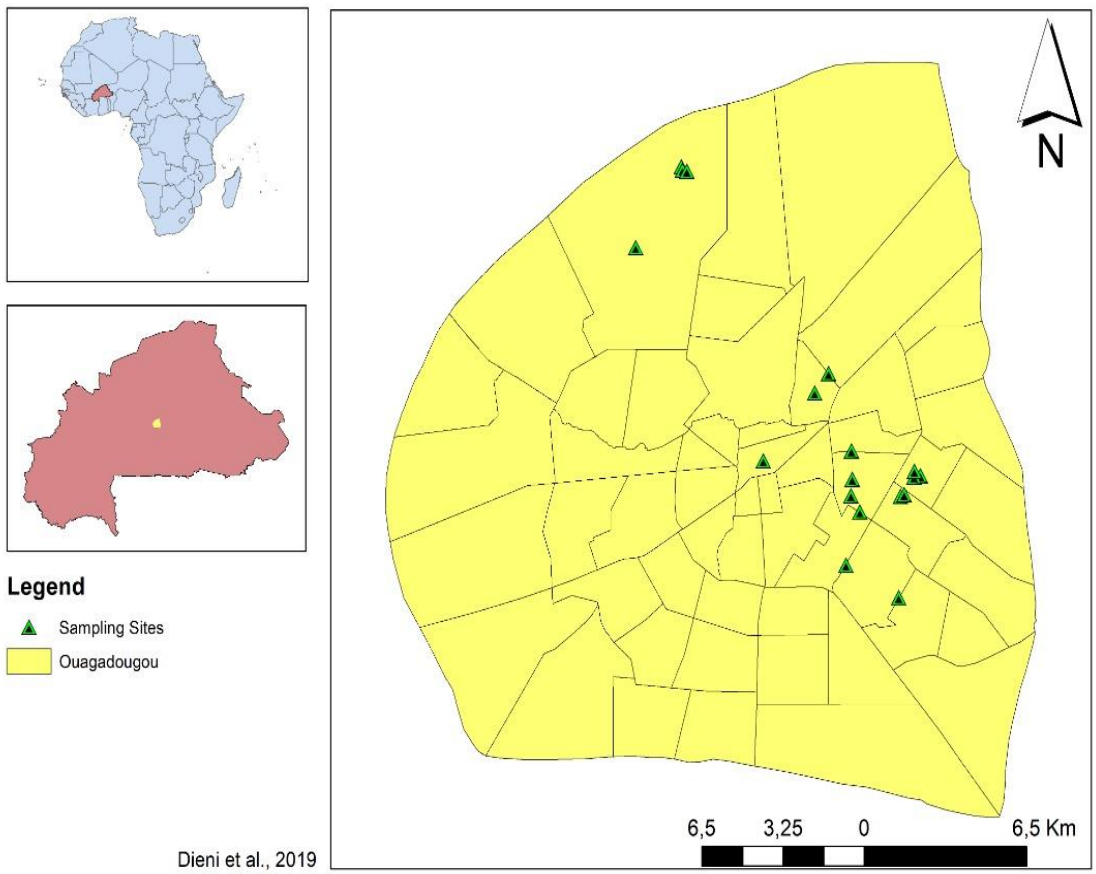

Figure 1: Sampling sites in Ouagadougou, Burkina Faso.
Source: Diéni, 2019.

RESULTS AND DISCUSSION

Presence of heavy metal in dough and *attiéké*

The contamination of dough and *attiéké* consumed in Ouagadougou by certain heavy metal are consigned in Table 1. In fermented cassava dough, the averages concentration of lead (Pb), Cadmium (Cd) and Aluminum (Al) were respectively 0.738 mg/kg, 0.006 mg/kg and 1.011 mg/kg, whereas concentrations in *attiéké* of lead, cadmium and Aluminum were respectively 0.393 mg/kg, 0.003 mg/kg and 0.492 mg/kg. A local foodstuffs production are processing to a variety of meals offering to population cheapest foods. Urbanization and social and economic situation increase street foods vending by several actors without no professional training in food safety (Soncy et al., 2015). These situations are very critical in developing country like Burkina Faso (Barro et al., 2006). Our results revealed amounts of lead were above the maximum level, which is set at 0.10 mg/kg (EC regulation, 2006) This contamination came certainly from soil and dust which increased the risk of contamination in an open environment (Nthenya et al., 2010). In Burkina Faso, using of chemical products in mining, industrial and agricultural activities are very developed and could lead to the contamination of water, air and soil with metallic trace elements as described by FAO (2018). Capo-Chichi et al. (2013) had shown that cassava roots consumed in Benin contained a high concentration of cadmium and lead. The concentrations of lead, cadmium and aluminum found in dough samples were higher than those found in *attiéké* due to the processing units (washing, pressing, fermentation etc.) due to a partial removal of metals during processing. Previous studies in Côte d'Ivoire and Benin have shown the presence of heavy metals such as lead, cadmium and aluminum in cassava products intended for the production of *attiéké* (Kouamé et al., 2019; Capo-Chich et al., 2019). These contaminations observed in *attiékés* sold in the cities of Côte d'Ivoire, Burkina Faso and between those obtained in this study could be explained by the level of unapplication of Good Hygiene Practices (GHP) in the production chain as pointed out by Capo-Chichi et al. (2013). Therefore, cassava dough and *attiéké* contaminated in this way can cause health concern for the consumer.

Microbiological parameters

Table 2 shows the results of microbial charge of fermented dough and *attiéké*. Only three (3) of the twenty (20) fermented cassava dough samples were contaminated by thermotolerant coliforms with an average bacterial load of $0.015x10^2$ $CFU.g^{-1}$. No *Salmonella*, was detected in dough, whereas 100% were contaminated by *Staphylococcus aureus* ($1.68x10^4$ $CFU.g^{-1}$ to $1.92x10^4$ $CFU.g^{-1}$). *Staphylococcus aureus* was counted at $1.05x10^4$ in Restaurants *attiéké* samples and at $2.96x10^4$ $CFU.g^{-1}$ in Producers samples. Then, no *attiéké* samples did not contain *E. coli* or *Salmonella*. The absence of *Salmonella* in *attiéké* could be due to its acidic nature and low pH as reported in Côte d'Ivoire by Kouamé et al. (2019. Therefore, the combined effect of organic acids produced during the fermentation period may possibly have an influence as bacteriostatic agents on spoilage microorganisms and pathogens (Sengun and Karapinar, 2012).

Most of food contamination occurs during processing. Fermented cassava dough and *attiéké* were contaminated by *Staphylococcus aureus*. This is probably due to human sources, by utensils, ambient air and personnel as mentioned by Kouamé et al. (2019). Barro et al. (2002) reported the major role of human and environment in the microbiological contamination of street-vended foods during processing dominated by manual operations. The high level of microbiological contamination of some samples with *S. aureus* exposes the consumer to foodborne pathogen infection such as toxi-infections and food poisoning (Kouamé-sina et al., 2019). According to Degnon et al. (2018), *Staphylococcus* is one most foodborne pathogens causing food poisoning due to enterotoxins produced by several species. These findings suggest awareness about *attiéké* consumption because these micro-organisms in foods, have negative impact on consumer health.

Table 1: Heavy metals composition of dough and *attiéké* samples.

Parameters	***Attiéké***					**Dough**
	Producers (n=5)	**Street-vended** (n=10)	**Restaurants** (n=11)	**Abgoville** (n=7)	**Agbodjama** (n=7)	**Vendors** (n= 20)
Lead (mg/kg)	3.116	4.451	3.256	4.221	4.619	7.379
Cadmium (mg/kg)	0.034	0.030	0.031	0.030	0.026	0.058
Aluminium (mg/kg)	3.198	5.603	5.065	5.147	5.596	10.105

Abgoville: Local *attiéké* (from processing units); Agbodjama: imported *attiéké* (from street-vended)

Table 2: Counting of microorganisms sought for dough and *attiéké* samples expressed $CFUg^{-1}$.

	Attiéké					**Dough**
Microorganisms	**Producers (n=5)**	**Street-vended (n=10)**	**Restaurants** (n=11)	**Abgoville (n=7)**	**Agbodjama (n=7)**	**Vendors (n= 20)**
Staphylococcus. aureus	$2.96x10^4$	$1.33x10^4$	$1.05x10^4$	$1.41x10^4$	$1.33x10^4$	$1.80x10^4$
Thermotolerant coliforms	00	00	00	00	00	$0.015x10^2$
Escherichia coli	00	00	00	00	00	00
Salmonella spp.	Abs	Abs	Abs	Abs	Abs	Abs

Abgoville: Local *attiéké* (from processing units); Agbodjama: imported *attiéké* (from street-vended); Abs: absence.

Conclusion

The study revealed *attiéké* sold in Ouagadougou has an unsatisfactory quality and their consumption could lead to diseases such as toxi-infection and poisoning. The loads of microorganisms and trace element content in *attiéké* are higher than the microbiological criteria. Major sources contributing to microbial contamination could be the personal hygiene of vendors, the place of preparation, time and temperature abuse of cooked foods, raw materials and utensils for cooking and serving. The results of this study suggest the application of preventive measures like good hygiene practices for *attiéké* sellers and producers to allow improvement of sanitary safety of this widely consumed food.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

ID is the instigator of this work, carried out laboratory analysis, data management and the manuscript writing. TSB, KAT, OZ, FT, BSRB, MEMN and AS have participated in the manuscript correction and data analysis. NB has done the Conceptualization, the supervision and review of this work. All authors have helped in revision and approved the final manuscript.

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