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Determination of ETM, Histamine and Mycotoxins in Garba, a Traditional Ivoirian Meal

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Abstract

The methods of preparation, conservation and sale of Garba, the traditional Ivorian street meal, abundantly consumed in Côte d'Ivoire, can be exposed to various infections resulting in the poisoning of consumers. The aim of this study was to evaluate the hygienic quality of Garba through the analysis and the determination of certain toxic chemicals. In three hundred (300) samples of Garba collected in four districts in Abidjan, toxic metals (cadmium, mercury, lead), biogenic amine (histamine) and mycotoxins (aflatoxins and ochratoxin A) were detected and quantified using official standardized methods. Different toxics analyzed were present at various levels in the Garba. The mercury, lead, cadmium and histamine levels in the Garba were respectively 0.19 mg/kg, 0.19 mg/kg, 0.03 mg/kg and 32.69 mg/kg. The detected mycotoxins included aflatoxins B1, B2, G1, G2 and ochratoxin A, with respective average proportions of 3.44 µg/kg, 1.90 µg/kg, 8.07 µg/kg, 0.56 µg/kg and 0.42 µg/kg. The mycotoxins levels in the Garba are higher than the recommended toxic levels, particularly the ones in aflatoxin B1 and G1. This suggests a sanitary risk associated with the consumption of this meal. Consequently, awareness campaigns and training of the Garba sellers in hygiene and a better regulation of this sector by the competent authorities are required.

Keywords

Garba, Toxic Metals, Biogenic Amine, Mycotoxins

1. Introduction

With the grip of sudden and unprecedented urban growth, and an increase in the size of the labor force, the demand for non-traditional services has gained momentum. Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in streets and other similar public places [1]. Street foods play an important socio-economic role, particularly in developing countries because they provide accessible and inexpensive food to the populations. Street vended foods are not only appreciated for their unique flavors, convenience and the role which they play in the cultural and social heritage of societies, but they have also become important and essential for maintaining the nutritional status of the populations [2] [3]. Besides offering business opportunities for developing entrepreneurs, the sale of street foods can make a sizeable contribution to the economies of developing countries.

Street foods raise serious safety concerns. Indeed contamination by chemicals and micro-biologicals contributes significantly to the occurrence of food borne illness [4]. The informal food production and marketing system is still strong in most countries, which presents challenges for enforcement of food safety regulations [5]. Street food vending has become an important public health issue and a great concern to everybody. This is due to widespread food borne diseases, due to the mushrooming of wayside food vendors who lack an adequate understanding of the basic food safety issues. Major sources contributing to microbial contamination are the place of preparation, utensils for cooking and serving, raw materials, time and temperature abuse of cooked foods and the personal hygiene of vendors [6]. Among other contamination reasons are conditions on public streets, with increasing levels of pollution due to dust and traffic. Furthermore street food can be contaminated by high concentrations of toxic chemicals such as pesticide residues, heavy metals, mycotoxins or unapproved food additives, such as textile colorants [4].

Food borne illnesses may result from the consumption of food contaminated by microbial pathogens, toxic chemicals or radioactive materials. Several analyses of street food samples showed heavy loads in total coliforms in some cases, and the presence of pathogenic bacteria such as *Salmonella spp.*, *Staphylococcus aureus*, *Clostridium perfringens* and *Vibrio cholerae [7]* [8]. While many food borne diseases may be self-limiting, some can be very serious and even result in death. In Côte d'Ivoire, "Garba" is a popular street food. It is a meal composed of *attiéké* (cassava meal) of 2nd grade [9], fried tuna, tomato, onion and fresh chili, seasoned with cooking broth. It is expected that, like other street foods, Garba is subject to various infections which can make it unsuitable for consumption. Indeed, its modes of production, preparation, preservation and marketing can be subjected to contamination factors and trigger food borne illness. To date, few scientific data on the hygienicquality of Garba is available.

The present study was undertaken to evaluate the hygienic quality of Garba. In order to achieve this, chemical molecules (heavy metals, mycotoxins, hista-

mine) that have been recognized to be toxic and as significant sources of food borne illness by the WHO, were investigated in several samples of Garba.

2. Material and Methods

2.1. Material

2.1.1. Garba Samples

The material for this study consisted of the collection of three hundred (300) samples Garba taken from Garba vendors located in four (4) municipalities of the city of Abidjan namely: Abobo, Cocody, Port-Bouet and Yopougon.

2.1.2. Solvents and Reagents

Nitric acid 65% (VWR chimicalsprolabo, France), Oxygenated water 30% (Carlo Erba Reagent, Espagne) were used for heavy metals mineralization. Trichloroacetic acid (Panareac, Espagne), Orthophtaldehyde (Merck, Germany), NaOH (VWR Chimicals Prolabo, France), HCl (Chimie plus Laboratoire, France) were used to prepare samples and histamine determination. For mycotoxin extraction, purification and quantification, Acetonitrile 99.9% (Prolabo, France), Méthanol 99.99% (Fischer Chimical/Angleterre), Hydrogénocarbonate de sodium (Prolobo/Belgique), Ultrapure Water (Corning/USA), Tampon phosphate salin PBS (OXOID/Angleterre), Acetic acid, *glacial,* 99% (Fluka analytical/Suisse), Toluene 99.9% (Carlo-chimie/Espagne) were used.

2.1.3. Apparatus

Atomic Absorption Spectrophotometer (Spectra AA 110, Varian), High Performance Liquid Chromatograph (Shimadzu LC 20 A Prominence) and High Performance Liquid Chromatograph (Shimadzu LC 20 AT) were respectively used for the analysis of heavy metals, histamine and mycotoxins.

2.2. Methods

2.2.1. Collection and Preparation of Samples

The collection of samples of "Garba" was done according to the Technical Guide developed by the Cofrac [10]. Samples were transported to the laboratory in coolers. Each sample weighted approximately 200 g and was made of attiéké (about 140 g) and tuna (about 50 g) accompanied by tomato, pepper and onion. Prior to the analysis, each sample was mixed and homogenized in a blender (Moulinex, France).

2.2.2. Analyses of Heavy Metals

For the examination of metals (mercury, lead, cadmium), the samples were mineralized for 30 to 45 min in a microwave digester (Milestone) using a nitric acid-oxygenated water mixture in closed containers under pressure. The dosage was done by atomic absorption spectrophotometry (Spectra AA110, Varian). The total mercury was determined by the flameless method according to the cold vapors principle [11] using a hydride generator (VGA 77). The lead and cadmium levels were measured by graphite furnace (GTA, Varian 110) of the

atomic absorption spectrophotometer on a mineral deposit obtained by sample digestion in a warm concentrated nitric acid-oxygenated water mixture (7/1) according to the AOAC method [12]. A Deuterium lamp was used for the correction of the background noise. For each series of analysis, a digestion of white and a sample of known reference concentration were analyzed to ensure quality control of the results. Readings were made with respect to a calibration curve drawn up in five points for each metal.

2.2.3. Analyses of Histamine

The determination of histamine was carried out by high performance liquid chromatography according to the Ifremer method [13]. The extraction was made using trichloro-acetic acid and detection by fluorimetry (RF-10AxL) at 360 nm and 450 nm as excitation wavelength and emission respectively after derivation using ortho-phtaldéhyde (OPA). The column used is a C18 column (Supelcosil LC 18, 15 cm × 4.6 mm, 5 mm).

2.2.4. Analyses of Mycotoxins

The determination of aflatoxins was performed by high performance liquid chromatography (Shidmadzu LC 20AT) on a purified extract and concentrated after derivation with trifluoroacetic acid by adaptation of the AOAC method [14] adapted for this study. The fluorimetric detection was ((RF-10AxL) at wavelengths of 365 nm and 435 nm, excitation and emission respectively. For the ochratoxin A, the method used was the standard NF EN [15]. The samples were treated with a polyethylene glycol solution and of sodium hydrogencarbonate, then filtered and purified on an immune affinity column. After elution with methanol, ochratoxin A was quantified by high performance liquid chromatography in reverse phase coupled with fluorimetric detection (λex = 330 nm and λém = 460 nm).

2.2.5. Methodsvalidation

Validation of the internal test methodswas made according to directive 2001/22/EC for heavymetals, adaptation of the proceduredeveloped by IFREMER of Nantes for histamine and directive 2002/26/EC formycotoxins

Data were expressed as mean ± standard deviation (SD). Data were analyzed by Anova at α = 0.05. Mean comparisons were made by the Fisher test and differentiation was considered significant at $p < 0.05$. The STATISCA 7.1 software is used for statistical analysis.

3. Results and Discussion

3.1. Analytical Quality Assurance

Analyticalresultsobtained on the materialused to validate the methods are presented in **Table 1**. The coefficients of variation (RSDR) are: 1.756 for Pb; 4.593 for Hg; 4.614 for the Cd for the repeatability test and 4.468 for the Pb; 4.529 for Hg; 4.809 for Cd for the reproductivity test.For repeatability tests,

oncentrations of the standard solutions were respectively 30 µl/L; 15 ul/L; 0.8 µl/L and 2.5 mg/L for Pb, Hg, Cd and histamine. For reproductivity tests, concentrations of standard solutions wererespectively 10 µl/l; 15 µl/l; 0.8 µl/l and 2.5 mg/L for Pb, Hg, Cd and histamine.

For mycotoxins, robustness of the analytical methods was examined and the results obtained are in Table 2.

3.2. SampleAnalysis

Mercury, lead, cadmium and histamine were measured in samples of Garba at average concentrations of 0.19 mg/kg, 0.19 mg/kg, 0.03 mg/kg and 32.69 mg/kg, respectively (Table 3).

The mycotoxins detected in the Garba samples had values ranging from 0.42 µg/kg (ochratoxin A) to 8.07 µg/kg (Aflatoxin G1) as shown in Table 4.

Among the detected aflatoxin, B2 aflatoxin was found in only 23.33% of the samples while aflatoxin B1 and OTA were found in more than half of the samples (Table 5).

3.3. Discussion

The present study was undertaken to evaluate the chemical quality of Garba. To

Table 1. Analytical results for Pb, Hg, Cd and histamine for validation methods.

	(% RSD)			
	Pb	Hg	Cd	Histamine
Repeatability	1.756	4.593	4.614	1.35
Reproductivity	4.468	4.529	4.809	4.07

Table 2. Analyticalresults for mycotoxins for validation Methods.

		Limits of detection (µg/Kg)	Limits of quantification (µg/Kg)	Accuracy (%)	Reliability (%)	Efficiency (%)	Standard deviations (%)
Ochratoxin A		0.05000	0.2000	5.67	0.26	86.0	6.01
Aflatoxin	B1	0.00564	0.0188	6.01	1.80	93.0	3.01
	B2	0.00151	0.0050	6.01	1.80	92.5	3.01
	G1	0.00136	0.0045	6.01	1.80	91.4	3.01
	G2	0.00143	0.0047	6.01	1.80	90.7	3.01

Table 3. Average levels of heavy metals and histamine in Garba samples.

Parameters (mg/kg)	Minimal Value	Maximal Value	Average Value
Mercury	0.01	0.40	0.19 ± 0.29
Lead	0.03	0.35	0.19 ± 0.20
Cadmium	0.01	0.04	0.03 ± 0.05
Histamine	13.61	66.10	32.69 ± 9.77

Table 4. Average levels of mycotoxins in Garba.

Parameters (µg/kg)	Minimal Value	Maximal Value	Average Value
Aflatoxin B1	0.02	35.78	3.44 ± 8.11
Aflatoxin B2	0.10	23.95	1.90 ± 5.68
Aflatoxin G1	0.56	69.32	8.07 ± 16.95
Aflatoxin G2	0.04	13.33	0.56 ± 2.43
Aflatoxin total	0.01	39.85	13.95±17.96
Ochratoxin A	0.06	1.83	0.42 ± 0.56

Table 5. Samples of positive Garba to mark mycotoxins.

Mycotoxins	Number of positive samples	Frequency (%)	Number of positive samples exceeding the authorized limit
Aflatoxin B1 *	170	56.60	40
Aflatoxin B2	70	23.33	-
Aflatoxin G1	140	46.66	-
Aflatoxin G2	120	40.00	-
Aflatoxins totals **	290	96.67	107
Ochratoxin A	190	63.30	-

*8 µg/kg Limit Value of the authorized Aflatoxin B1 (CE/1881/2006), **15 µg/kg Limit Value of the total authorized Aflatoxin.

achieve the purpose, chemical molecules were investigated in several samples of Garba. The chemical analyzes of the samples of Garba showed that it contains heavy metals, but also histamine as well as mycotoxins. At the level of heavy metals, namely lead, cadmium and mercury, average proportions obtained were, however, lower than the recommended limits (1 mg/kg for mercury, 0.3 mg/kg for lead and 0.1 mg/kg for cadmium) in accordance with standard CE 1881 [16]. The average mercury content in the Garba samples was indeed 0.19 mg/kg against 1 mg/kg representing the limit value of this toxic in fish, recognized as the main source of food contamination due to mercury. Garba being a fish-containing food (tuna), the presence of mercury in the Garba could be attributable to this component. Fish, including tuna, is indeed recognized for his stacking character for mercury, the latter tend to remain stored in different organs [17]. In this regard, several authors have reported the presence of mercury in fish of the Scombridae family [18] [19] [20].

The detected lead and cadmium in the samples analyzed could in turn come from several Garba components such as fish and vegetables. Indeed, like fish, vegetables such as onions and tomatoes are also frequently involved in the contamination due to these two metals [21] [22] [23]. The absence of a limit value for lead in Garba does not give any indication of the quality of the Garba dish for this metal. It should, nevertheless, be noted that some lead values obtained in the Garba samples are relatively high (0.34 and 0.35 mg/kg) compared to the fish

limits values. At the cadmium level, the mean value of 0.03 mg/kg, was small compared to the limit value of 0.1 mg/kg applied to potato that could be compared to cassava, which is the raw material serving for the production of the main ingredient of the Garba namely *attiéké*. Based on this comparison, the level of cadmium contamination Garba appeared to be acceptable.

The mean levels of lead and cadmium found in the Garba samples were below the tolerable limit values that are respectively 0.19 and 0.03 mg/kg. However, among all samples analyzed, some contained values above these limits. Garba consumption containing high levels of lead and cadmium could cause physiological disorders among consumers. Lead is recognized as responsible for lead poisoning, an acute or chronic professional or domestic poisoning. It is a serious disease that can affect humans and particularly children and pregnant women [24]. Conversely, cadmium causes lung problems if inhaled. It can be very harmful to low concentration when consumed over a long period [25].

Concerning histamine, its content in the analyzed samples (66.1 mg/kg) did not exceed the tolerated limit value in tuna (100 mg/kg). Potential sources of this biogenic amine in the Garba could be fish, vegetables but also *attiéké*. Many authors have indeed shown the presence of histamine in tuna [26] [27] [28] and tomato [29]. Moreover, *attiéké* could be implicated because of its production process mainly based on fermentation. It has indeed been shown that many fermented foods (cheese, drinks, sausages and vegetables) contained histamine and their consumption may have had toxicological consequences [30] [31].

All the current mycotoxins in the samples were detected, suggesting a fungal contamination of this meal. Several studies have already mentioned the presence of aflatoxins in food. It was the case of Muthomi *et al.* [32], Kang'e the and Lang' at [33], Offifahand Adesiyun [34] and Lewis *et al.* [35] that have detected the presence of aflatoxins in pasteurized products, cheese, peanut butter, alcoholic beverages from cereals, foods for infants and maize. In the current study, more than half of the samples contained aflatoxin B1. This mycotoxin is deemed extremely dangerous to humans and is responsible for liver cancer. Indeed, aflatoxin is the basis of the acute destruction of the liver and cirrhosis of the liver, and the development of tumors or other genetic defects [36] [37] [38].

The aflatoxin B1 contents of the Garba samples were between 0.02 and 35.78 μg/kg. The maximum value for the aflatoxinB1 found in samples of Garba was much higher than the regulatory limit value of 8 μg/kg [16]. Ingestion of high aflatoxin B1 could cause, as well, liver cancer [39] [40] and growth delay in children [41]. Thus the consumption of Garba could be source of disease among consumers. The presence of aflatoxin B1 in the Garba could probably be due to the *attiéké*. *Attiéké* contains a relatively high humidity, over 40%, [9] [42] [43] and is expected to be contaminated with mold. Previous studies have already mentioned the presence of mold in *attiéké* [44] [45] [46].

Acceptable levels of mycotoxins in food are not regulated in Ivory Coast. However, in countries or in within the European Community where they are,

they vary depending on the type of mycotoxin and the food. Under the EU Regulation [16], which defines the maximum permissible quantities of mycotoxins in foodstuffs, the highest values are 15 µg/kg for the sum of the 4 aflatoxins, against 8 µg/kg for aflatoxin B1. Our findings are disagreeing with those of [47] [48] that have not detected the presence of aflatoxins (B1, B2, G1 and G2) in analyzed samples of attiéké during their Experiments. In our study, only thirty Garba samples (10%) were completely free of these toxins.

The level of aflatoxin B1 in the analyzed samples were between 0.02 and 35.78 µg/kg with an average value of 3.43 µg/kg. Forty Garba samples (23.52%) contaminated with mycotoxins had values that exceeded the regulatory limit (8 µg/kg), which could be dangerous for Garba consumers. In France, according to the 2001-2004 Monitoring Plan, the average concentrations of aflatoxins cereals are between 0.2 and 0.3 µg/kg. This includes only three products (rice, semolina and maize flour) where the concentrations of aflatoxin B1 exceed the regulatory limit (2 µg/kg). The average of aflatoxin B1 in all the food was 5.31 µg/kg [49].

In relation to the total aflatoxin content (37%) the analyzed samples show significant levels exceeding the regulatory limit (15 µg/kg). Furthermore, the average value of the sum of the aflatoxins in the Garba (39.27 µg/kg) was higher compared to the fixed limit value of 15 µg/kg. This situation suggests that Garba could be a dangerous food for the health. This was as well reported for other foods containing high levels of aflatoxin [50]. They have shown that peanuts in Morocco were highly contaminated with mycotoxins, while they had a total aflatoxin content of 850 µg/kg.

The authorized quantities of OTA in food ranged from 2 µg/kg to 10 µg/kg [16] depending on the nature of the food. The amounts of OTA determined in the Garba samples did not exceeded 2 µg/kg. This was as well reported by [47] in a similar experiment where traces of OTA were not exceeding 0.2 µg/kg in the samples of *attiéké*. In Côte d'Ivoire, data on OTA vary depending on the nature of the analyzed products. Dano *et al.*, [51] reported that 63% of the coffees analyzed in their experiments, contained a value of OTA above 20 µg/kg with a mean value of 31.3 µg/kg. In maize, Sangaré *et al.* [52] evaluated the OTA between 3 µg/kg and 1738 µg/kg against 119 µg/kg in the same matrix [53]. According to a study conducted between 1998 and 2002, millet, rice and peanuts contained contents between 17 and 204 µg/kg, between 9 and 92 µg/kg and 0.6 to 64 µg/kg respectively [52].

Elsewhere in Morocco, Tantaoui-Elaraki *et al.* [50] reported on the outcome of their experiments, that a significant proportion of barley samples (36%) contained no OTA. For the samples containing OTA, those were evaluated between 1.13 and 2.83 µg/kg. The presence of OTA in particular, and in general those of mycotoxins in these foods could be explained by their high humidity, favorable condition for the development of molds such as *Aspergillus*, *Penicillium* and *Fusarium* responsible for the production of these toxins [54].

4. Conclusion

Ensuring food safety requires due attention during harvest, transport, processing, storage and finally during food preparation and storage by consumers. The Garba Samples analyzed contained mycotoxins and heavy metals. If heavy metals and histamine were found at low levels not exceeding the recommended threshold values by the Food Standards, mycotoxins were detected at levels beyond the recommended limits. The consumption of Garba could be a source of food poisoning for consumers. Application of sound policies and regulations should be advocated in Côte d'Ivoire to ensure public health and safety. Awareness campaigns and training of the Garba vendors in hygiene and a better regulation of this sector by the competent authorities are required.

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