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Evaluation of practices favoring the development of mycotoxigenic molds in rice sold in the retail markets of Abidjan, Côte d'Ivoire

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Mycotoxins constitute a real problem of public health because of their highly toxic effects for the populations. This study aims to evaluate the practices that favor aflatoxins and ochratoxin A development in rice sold on the markets of Abidjan (Cote d'Ivoire). A food consumption survey was conducted among 45 traders and 135 consumers of rice in three markets in Abobo and Cocody communes. A simple, open-ended questionnaire was submitted to the rice traders and consumers. It covered socio-demographic characteristics (age, sex, level of education), location and conditions of storage, place of supply, and knowledge of mycotoxins. Following the survey, 20 samples of rice, prized by the population, were collected and analyzed. Total aflatoxin and ochratoxin A levels were determined using HPLC coupled with mass spectrometry. The results revealed that all traders were unaware of the existence of mycotoxins. The storage and marketing of rice are housed in very unhygienic premises (lack of brick walls in some cases, poor quality tin roof, premises not swept regularly). Most traders renew their rice stocks over an average of one to two months (66.66% in Abobo; 79.99% in Cocody) and prefer to buy their supplies from large stores and other markets in Abidjan. Aflatoxin B1 (0.75±0.05 µg/kg) and total aflatoxin (1.73±0.34 µg/kg) were detected in the rice samples from Abobo market. The presence of Aflatoxins in rice could be related to poor post-harvest practices identified.

Key words: Rice, total aflatoxins, ochratoxin A, practices, Abidjan.

INTRODUCTION

Mycotoxins are secondary metabolites from several species of molds belonging to the genera *Penicillium*, *Aspergillus* and *Fusarium* (AFSSA, 2009; Wacoo et al., 2014). These molds grow on several food products mainly peanut and cereals such as corn and rice (Park et al., 2005; Reinhold and Reinhardt, 2011). Mold growth and mycotoxin secretion can occur before, during, and after harvest and are related to a range of moisture and

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Table 1. Distribution of rice samples from different markets.

Most consumed rice	Number of samples per site			Weight of each sample (g)	Number of samples
	Abobo	Cocody	Supermarket		
Thai broken rice (TBR)	4	5	-	1000	9
Vietnamese long grain rice (VLR)	-	2	2	1000	4
Vietnamese broken rice (VBR)	2	2	-	1000	4
Indian Malo Woussou Rice (IMR)	3	-	-	1000	3
Total	9	9	2	-	20

temperature conditions (Mateo et al., 2002; Alborch et al., 2011). The Food and Agriculture Organization of the United Nations (FAO) estimates that approximately one quarter of the world's agricultural production is contaminated by mycotoxins, representing an economic loss of 5 to 10% (FAO, 2004). In addition to the economic impact, mycotoxins pose a real public health problem due to their chronic toxicity (carcinogenic, mutagenic, teratogenic, nephrotoxic, estrogenic, immunosuppressive) related to repeated ingestion of low doses (Turner et al., 2003; IARC, 2012).

In Côte d'Ivoire, rice is the staple food for both rural and urban populations with an estimated average consumption of 58 kg/inhab/year (FAO, 2010). Each year, Côte d'Ivoire imports about 1.3 million tons of rice to meet the population's growing food needs (PAM, 2019). The rice trade is carried out by several actors, including importers and distributors, including wholesalers, semi-wholesalers and retailers. The role of each of these actors in the sanitary quality of the rice consumed is decisive, because the risk of fungal contamination at each stage of the distribution chain is real. The levels of mycotoxin contamination in rice in Côte d'Ivoire are sometimes higher than the maximum threshold set by the European Commission, which is 2 µg/kg for aflatoxin B1 (AFB1), thus putting consumers at great risk (Sangare-Tigori et al., 2006; Fofana et al., 2019). Controlling the critical points of fungal contamination in the rice marketing chain is proving to be an essential element in the fight against mycotoxins. It is in this perspective that this study aims to assess the practices that may be at the origin of the development of aflatoxin and ochratoxin A molds in rice sold in some retail markets of the city of Abidjan in Côte d'Ivoire.

MATERIALS AND METHODS

Consumption survey

A rice consumption survey was conducted in two (2) communes in the district of Abidjan, namely Abobo and Cocody, from August 20th to September 20th 2019. These two communes were chosen because of their cosmopolitan character, their high population density and their social and food diversity. Three (3) markets per commune were chosen for the survey. In total, forty-five (45) rice traders and one hundred and thirty-five (135) consumers per commune were included in this study. A simple, open-ended questionnaire was submitted to the rice traders and consumers. It covered socio-demographic characteristics (age, gender, education level), the rice most purchased, storage location and conditions, procurement location, and knowledge of mycotoxins.

Determination of aflatoxin and ochratoxin A concentration in rice

Sampling and sample preparation

The sampling of rice was carried out according to the European Commission Regulation No 401/2006 of 23 February 2006 (CE, 2006) laying down the sampling methods and the methods of analysis for the official control of the levels of mycotoxins in foodstuffs. Samples of imported rice most consumed by the population in each locality were selected at random. In total, twenty (20) elementary rice samples of about 1000 g each were collected from six markets (3 in Abobo and 3 in Cocody) and two supermarkets in Cocody for the determination of mycotoxins. It is about 9 samples of Thai broken rice (TBR), 4 samples of Vietnamese long grain rice (VLR), 4 samples of Vietnamese broken rice (VBR) and 3 samples of Indian Malo woussou rice (IMR) (Table 1). Thus, 9 samples were taken in the markets of Abobo, 9 in the markets of Cocody and 2 in the supermarkets of Cocody (Table 1). The collected rice samples were packaged in bags and sent to the laboratory for analysis.

Extraction and purification of aflatoxins and ochratoxin A from rice

The extraction, purification and quantification of aflatoxins and ochratoxin A were performed according to ISO 16050/2003 method (ISO 16050/2003, 2003). In a centrifuge tube, 20 g of finely ground rice were introduced. To this was added 20 ml of the mixture water-acetonitrile (50/50; v/v). The whole was homogenized with a magnetic stirrer for 30 min. After 30 min of stirring, 4 g of magnesium sulfate ($MgSO_4$), 1 g of sodium chloride (NaCl), 1 g of nitrate (NO_3) and 0.5 g of sodium hydride (Na2H) were added. The mixture was then centrifuged at 3500 rpm for 5 min by a zentrifugen Rotofix 32 A Hehich (Germany) centrifuge and the supernatant was collected and filtered through a 15 mm/0.22 µm nylon filter.

Quantification of aflatoxins and ochratoxin A in rice

A volume of 100 µl of extract was taken and put in a 1.5 ml vial and filled to the mark with methanol. The content of the vial was analyzed by High Performance Liquid Chromatography (Agilent 1200 series technology, USA) with an automatic injector coupled to

Table 2. Socio-demographic characteristics of rice traders in Abobo and Cocody communes.

Variable	Sub-variable	Communes			
		Abobo		Cocody	
		Number	%	Number	%
Gender	Male	41	91.1	40	88.89
	Female	4	8.9	5	11.11
Education level	Non enrolled	38	84.44	29	64.44
	Primary	7	15.56	11	24.44
	Secondary	0	0	5	11.12
Knowledge of Aflatoxins and Ochratoxin A	Yes	00	0	0	00
	No	45	100	45	100

mass spectrometry (LC/MS/MS). Chromatographic measurements were performed at 40°C at a flow rate of 0.4 ml/min. The mobile phase consisted of a water-methanol mixture (50: 50, v/v). The stationary phase had the following characteristics: colum LC phenomena synergy fusion 80 Å, length 150 mm, internal diameter 3 mm and particle radius 4 µm. The injection volume was 30 µl. The flow rate of the mass spectrometer was maintained at 0.4ml/min. The aqueous phase A was water and the organic mobile phase B was methanol. Both phases contained 0.5% formic acid and 5 mmol ammonium acetate. The limits of detection and quantification were 0.04 and 0.4 µg/kg, respectively.

Statistical analysis

The means of each aflatoxin and ochatoxin A concentration were compared through one-factor analysis of variance (ANOVA) with Statistica version 7 statistical software. Significance of the analysis is tested by comparing the P probability at the α threshold of 0.05 with Duncan's test. Survey data were processed with Excel version 2016 software.

RESULTS

Consumption survey

Socio-demographic characteristics of rice traders in the communes of Abobo and Cocody

Table 2 presented the socio-demographic characteristics of rice traders in the communes of Abobo and Cocody. Indeed, the survey revealed that rice sales are predominantly a male activity, with 91.7 and 88.89%, respectively in the communes of Abobo and Cocody. The majority of traders in the two communes were illiterate, with a rate of 84.44% in the commune of Abobo and 64.44% in the commune of Cocody. The rate of school attendance was very low, at 15.56% in the commune of Abobo and 35.56% in the commune of Cocody. Among those who had attended school in the commune of Cocody, 24.44% had primary education and 11.12% had secondary education. In contrast, none of the people surveyed in the commune of Abobo had a secondary education. In both communes, all traders (100%) had no knowledge of the existence of mycotoxins in rice.

Most consumed rice and frequency of consumption in the communes of Abobo and Cocody

The most consumed rice and the frequency of rice consumption are shown in Table 3. Most consumed rices in the two communes were Thai broken rice, Indian malo woussou rice and Vietnamese broken rice. Thai broken rice had accounted for more than half of the sales with 51.11 and 53.33%, respectively in the communes of Abobo and Cocody. The majority of consumers (more than 60%) in both communes had consumed rice at least once a day. All traders and consumers (100%) had no knowledge of aflatoxins and ochratoxin A.

Characteristics of the rice storage site in the communes of Abobo and Cocody

Characteristics of the rice storage site in the two communes are shown in Table 4. All the stores of the storage sites had a roof covered with sheet metal (100%). In the commune of Abobo, the walls of the stores were made of either sheet metal (57.78%) or brick (42.22%), while all the walls in Cocody were made of brick (100%). The floors in these stores were mostly made of cement (75.55% in Abobo and 53.33% in Cocody), or tiles (20% in Abobo and 37.78% in Cocody) or concrete (4.45% in Abobo and 8.89% in Cocody). Only sweeping was used to keep the storage environment clean in the both communes. The majority (88.89% in Abobo; 91.11% in Cocody) had swept their premises at least once a month.

Table 3. The most consumed rice, frequency of consumption in the communes of Abobo and Cocody.

Variable	Sub-variable	Communes			
		Abobo		Cocody	
		Number	%	Number	%
Most consumed rice	TBR	69	51.11	72	53.33
	IMR	36	26.67	14	10.37
	VLR	22	16.3	32	23.7
	Others	8	5.8	17	12.6
	Total	135	100	135	100
Frequency of consumption	1-2 times/day	97	71.85	84	62.22
	1-4 times/week	14	10.37	17	12.6
	More than 4 times /week	24	17.78	34	25.18
Knowledge of Aflatoxin and Ochratoxin A	Yes	00	00	00	00
	No	135	100	135	100

Thai Broken Rice (TBR), Vietnamese Long grain Rice (VLR), Vietnamese Broken Rice (VBR), Indian Malo Woussou Rice (IMR).

Table 4. Characteristics of the rice storage site in Abobo and Cocody communes.

Variable	Sub-variable	Communes			
		Abobo		Cocody	
		Number (n)	%	Number (n)	%
Nature of the roof	Sheet metal	45	100	45	100
Nature of the wall	Sheet metal	26	57.78	0	0
	Brick	19	42.22	45	100
Type of floor	Cement	34	75.55	24	53.33
	Tiles	9	20	17	37.78
	Concrete	2	4.45	4	8.89
Sweeping of the floor	Yes	45	100	45	100
	No	00	00	00	00
Frequency of sweeping	1 time/month	40	88.89	41	91.11
	2 times/month	3	6.67	3	6.67
	3 times/month	2	4.44	1	2.22
Floor washing	Yes	00	00	00	00
	No	45	100	45	100
Floor disinfection	Yes	00	00	00	00
	No	45	100	45	100

Distribution of stocking duration, place of supply and number of suppliers

Table 5 shows distribution of stocking duration, place of supply and number of suppliers. In both communes, most traders had renewed their stocks after an average period of one to two months (66.66% in Abobo and 79.99% in Cocody). Stocks that were renewed after more than two

Table 5. Distribution of stocking duration, place of supply and number of suppliers in Abobo and Cocody communes.

Variable	Sub-variable	Communes			
		Abobo		Cocody	
		Number (n)	%	Number (n)	%
Stocking duration (months)	<1	9	20	5	11.11
	1 to 2	30	66.66	36	79.99
	>2	6	13.34	4	8.9
Place of supply	Adjamé	5	11.11	30	66.67
	Department store	40	88.89	12	26.66
	Supermarket	00	00	3	6.67
Number of suppliers	1-2 suppliers	23	51.11	10	22.22
	>2 suppliers	22	48.89	35	77.78

Table 6. Aflatoxin and ochratoxin A concentration in the analyzed rice.

Mycotoxins (%)	% positive samples (n=20)	Mean concentration (µg/kg)	Range (µg/kg)
Aflaotxin B1	10	0.75 ±0.05	0.45 -1.17
Totals Aflatoxins	10	1.73±0.34	0.65-2.01
Ochratoxin A	00	ND*	-

*: No determined.

months represented 13.34 and 8.9% in Abobo and Cocody, respectively. In the commune of Abobo, merchants had preferred to buy their supplies from department stores (88.89%). In contrast, traders in the commune of Cocody had bought their supplies from the Adjamé market (another commune in the Abidjan district) (66.67%) and department stores (26.66%). Each merchant was bought from several suppliers. A large proportion of traders (48.89% in Abobo and 77.78% in Cocody) had more than two suppliers.

Aflatoxin and ochratoxin A concentration

Aflatoxin and ochratoxin A concentration is shown in Table 6. Aflatoxins were found in two of the three samples of Indian malo woussou rice taken in Abobo commune. This represents 10% of positive samples for all rice samples analyzed. The total aflatoxin content was 1.73 and 0.75 ±0.05 µg/kg for aflatoxin B1. Ochratoxin A was not found in any of the rice samples analyzed.

DISCUSSION

Post-harvest development of mycotoxigenic molds in foodstuffs occurs during the processes of drying, storage, handling, transportation, and marketing (Mandeel, 2005; AFSSA, 2009; Manizan et al., 2018). In this study, the conditions conducive to the development of aflatoxins and ochratoxin A in imported rice during storage and marketing operations at retailers were assessed. According to the surveys, the storage and marketing of rice are housed in very unhygienic premises (no brick walls, poor quality tin roof, premises not swept regularly). These storage conditions are responsible for the humidification of the rice, especially in the rainy season, and for its infestation by insects. Mycotoxin formation is influenced by physical factors that include temperature and humidity. In general, the optimum conditions for aflatoxin production are a water activity of 0.99 and a temperature of 33°C (Ominski et al., 1994; Puschner, 2002). Rice is a foodstuff that can easily absorb water from humid air through poor quality sheets and walls. This ease of water absorption increases water content and thus susceptibility to fungal contamination and production of mycotoxins including aflatoxins (Nguyen, 2007). The relatively long storage time of rice (two months or more) in makeshift premises at most traders' is a factor conducive to fungal development and therefore mycotoxin secretion. Indeed, the grains can be altered during this period by insects, rodents and mites, which favors the development of molds on the stocks. Moreover, insects, far from playing a minor role in the contamination of food in storage areas, are themselves vectors for certain fungal spores (Pfohl-Leszkowicz, 1999; Hubert et al., 2007).

Rice sellers are predominantly males with low levels of education who have no knowledge of mycotoxins and their effect on consumer health. This lack of knowledge about mycotoxins is a risk factor. Indeed, rice is the best substrate for the development of aflatoxin-producing mold species. The low level of education, the ignorance of mycotoxins associated with unhealthy conditions can only favor their development in rice. The traceability of imported rice is complex and involves different actors, including importers and distributors (wholesalers, semi-wholesalers and retailers).

Retailers obtain their supplies from various suppliers residing in several communes, including large storage stores and supermarkets. Hygienic conditions, especially at the store level, could also be similar to those encountered at the retail level, also favoring the development of molds.

These hygiene failures in the industry are exacerbated by the absence of government structures such as food safety programs that routinely use information on factors that promote fungal contamination to establish prevention and control procedures to provide consumers with safe and wholesome food. In order to assess mold growth in imported rice, certain mycotoxins, including total aflatoxins and ochratoxin A, were tested in four of the most popular imported rice grades.

Aflatoxins were found in 10% of the total samples of rice sold in the locality of Abobo with an average level of 0.75 ±0.05 µg/kg for aflatoxin B1 and 1.73± 0.34 µg/kg for total aflatoxins. In this low-income locality, the premises housing the rice trade are much more unsanitary than those in Cocody, where the premises are relatively better constructed. This could partly explain the presence of aflatoxins in imported rice sold in the commune of Abobo. It cannot be ruled out that this aflatoxin contamination of rice predates storage and marketing at retailers. The mycotoxigenic molds could well develop in the rice during storage at wholesalers and semi-wholesalers. However, the lack of knowledge of mycotoxins by the actors involved in the retail rice trade, coupled with poor hygienic practices and conditions, contribute to the exacerbation of fungal contamination of foodstuffs.

Contamination of foodstuffs in general and rice in particular by aflatoxins in Côte d'Ivoire is recurrent and is reported by several authors (Sangare-Tigori et al., 2006; Fofana-Diomandé et al., 2019). Admittedly, the aflatoxin values found in this study are below the maximum threshold of 2 µg/kg for AFB1 set by the European Commission N° 1881/2006 (CE, 2006). However, aflatoxin B1 is a carcinogenic substance for humans, so the prolonged exposure of the population to this mycotoxin poses a great risk to their health. It is not excluded that this contamination of rice predates storage and marketing operations. However, the lack of knowledge of mycotoxins by the actors involved in the retail rice trade, combined with poor practices and poor hygienic conditions, contribute to the exacerbation of fungal contamination of foodstuffs. In this study, ochratoxin A was not detected in any of the rice samples analyzed despite the favorable conditions for its production by molds. These results could be explained by the fact that rice is not a good substrate for the development of ochratoxinogenic molds. These results corroborate those of Kouadio (2012) who suggested that human exposure to Ochratoxin is relatively low in Côte d'Ivoire.

Conclusion

The study showed that in the communes of Abobo and Cocody, the rice sales and storage areas were neither washed nor disinfected by the traders. Sweeping was rarely done. The analyses of collected rice had shown that some of rice simples were contaminated with aflatoxins. This contamination could be linked to the poor hygienic conditions observed in the premises where the rice is sold at retail. Aflatoxins being carcinogenic for human, regular exposure of the population to these mycotoxins, even at low doses, could have serious consequences on consumer health. It is therefore incumbent on the competent authorities to raise awareness of good practices among all actors involved in the rice trade in order to ensure a healthy diet, free of microbial contamination, for consumers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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