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Assessment of Exposure to Ochratoxin – A (OTA) through Ground Roasted Coffee in two Cameroonian Cities: Yaounde and Douala

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Abstract Occurrence of ochratoxin A (OTA) in ground roasted coffee from different brands and types available in Yaounde and Douala cities was assessed. Based on these data, exposure of the Cameroonian population to OTA through coffee consumption was estimated. Coffee samples were purchased in hypermarkets and supermarkets of the two selected cities of Cameroon. OTA was extracted, cleaned-up by immunoaffinity columns, and detected by HPLC-fluorescence detection. OTA was found in 64.7% of pure Arabica coffee, 75% of pure Robusta coffee, and 62% of Arabica / Robusta mixture coffee, with OTA levels ranging from 0.3 to 4.9 $\mu g.kg^{-1}$, 0.6 to 18 $\mu g.kg^{-1}$ and 0.3 to 7.4 $\mu g.kg^{-1}$ respectively. Robusta Coffee brands are the most contaminated, follow by coffee mixture brands and Arabica coffee brands. The daily intake of OTA is 0.065 ng / kg bw per day. Consumption of OTA from coffee in the cities of Douala and Yaoundé are then very low compared to that of Europe who is 2-3 ng/kg bw per day. Consumption of OTA from coffee in the cities of Douala and Yaounde is very low compared to that of Europe, for which the rate is 2-3 ng/kg bw per day.

Keywords Ochratoxin-A, Immunoaffinity Columns, HPLC, Coffee Brands, Ground and Roasted Coffee, Daily Intake

1. Introduction

Ochratoxin A (OTA) is a mycotoxin that can be found in several foodstuffs, such as cereals and derived products, maize, beer, wine, grape juice, **dried vine fruit**, **dried grapes**, spices, cocoa and coffee. Ochratoxin A presence has been also detected in lower amounts in meat, eggs and milk as a product of the carryover of animals fed with contaminated feedstuffs. Typical target organs of OTA are kidney and liver; OTA residues have also been found in significant amount in human milk, during the breastfeeding period, and in blood as excretion product[1, 17, 18, 19, 23, 24].

This toxin is produced in food by certain species of the *Penicillium* and *Aspergillus* genera[15, 21]; **with** the latter being the most frequent producers in coffee.

More recently, the European Food Safety Authority (EFSA) was asked by the Commission to produce an opinion aimed at updating the risk assessment on OTA taking into account new scientific information. In the opinion, published in April 2006, EFSA states that OTA is nephrotoxic in all animals' species tested and exerts immunotoxic, neurotoxic and teratogenic effects at high dose levels[8]. The evidences of the renal toxicity are associated with cellular oxidative stress. The observed effects are all dose and time dependent.

The World Health Organization/Food and **Agricultural Agriculture** Organization Joint Expert Committee on Food Additives[14] has set in 2007 a Provisional Tolerable Weekly Intake (PTWI) of 100 ng/kg bw/week, retaining this value from previous OTA evaluations[12, 13], whereas the European Food Safety Authority (EFSA) Scientific Panel on Contaminants in the Food Chain established a PTWI of 120 ng/kg bw/week[8]. JECFA and EFSA PTWI values correspond to the Provisional Tolerable Daily Intake (PTDI) values of 14 and 17ng/kg bw/day, respectively.

Concerning coffee contamination, the European Union has set the OTA maximum level for roasted coffee beans and ground roasted coffee at 5 ng/g, whereas for instant coffee the limit was established at 10 ng/g[9].

In the Cameroonian market, several kinds of coffee are available: mainly ground roasted and instant, and the decaffeinated. Three types of varieties can be found: Arabica, Robusta and mixture of both in different proportions.

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The aim of this study was to assess the occurrence of OTA in coffee from different brands and types available in Cameroon, and to assess the exposure to OTA through coffee consumption particularly in the adult population of Yaoundé and Douala cities.

2. Materiels and Methods

2.1. Sampling

Analyzes have focused on different brands of roasted and ground coffee found in markets and supermarkets in the cities of Douala and Yaoundé. Maximum brand of coffee (Arabica, Robusta and mixture) that are available, whatever the origin (foreign or local) were purchased. A total of 17 brands of pure Arabica coffee, 16 brands of pure Robusta coffee and 16 brands of Arabica / Robusta mixture coffee were found. Three samples (packages of 250 g each), when available, were taken randomly in six markets and supermarkets per city. A total of 147 coffee samples were analyzed.

2.2. Coffee Drink Preparation

The coffee drink was prepared according to the "filter" method. The concentration was 6 g of ground coffee for 100 ml of water at 90-100°C.

2.3. OTA Quantification

The official method (method of the European Union) for the determination of ochratoxin A was based on the method published by Pittet et al in 1996[26] for soluble coffee, which is an adaptation of the method developed by Nakajima et al in 1990[22].

OTA was extracted from 10g of coffee using a solvent (methanol / sodium bicarbonate at 3% V/M) for 30 min at room temperature.

After filtration, 10 ml of the extract were diluted with 30 ml of PBS phosphate buffer at pH 7.3) and were purified on the immunoaffinity column. The extract was introduced into the immunoaffinity column where there was uptake of OTA by antigens. The column is then rinsed with PBS to release the foreign and non specific components. The toxin was eluted from the column with methanol.

The mixture was then evaporated under a stream of nitrogen at 70°C and the elute was collected in a beaker, and was dissolved in 1 ml of mobile phase and was quantified using HPLC.

The operating conditions are as follows: injection loop 100 ml, HPLC column C18 reverse phase, ODS 1 to 5 microns with precolumn thermostat at 35°C. The pressure is between 95 to 105 bars. Isocratic flow is 1mL/min. Detection is done by fluorimetry. The excitation wavelength is 333 nm and that of emission is 460 nm. The retention time of OTA is approximately 12.6 min.

3. Results and Discussion

3.1. Distribution of Samples by City

Table 1 below shows the distribution of coffee samples and coffee brand by city. Analyzes were conducted on 49 coffee brands of roasted and ground coffee, i.e. 147 coffee samples (74 samples in Douala and 73 samples in Yaoundé). It comes to 17 brands of pure Arabica coffee (7 of Cameroonian origin marks and 10 marks of foreign origin), 16 pure Robusta coffee brands (7 Cameroonian brands and 9 foreign brands) and 16 Arabica / Robusta mixture brands (7 of Cameroonian origin marks and 9 foreign brands).

Table 1. Distribution of samples by city

	Brand	City of Douala	City of Yaoundé
Pure Robusta coffee	Local	12	9
	Foreign	12	15
Pure Arabica coffee	Local	10	11
	Foreign	15	15
Arabica/Robusta mixture	Local	10	11
	Foreign	15	12
Total		**74**	**73**

3.2. OTA Occurrence in Ground Roasted Coffee

3.2.1. OTA in Ground Roasted Coffee

Out of 51 samples of ground roasted coffee of pure Arabica, 33 were positive, i.e., 64.7% of the samples presented contamination values above the LOD of the method (Table 2). The positive levels ranged from 0.3 to 4.9µg.kg^{-1}: value which is below the limit set by the European Union (5 ppm). The mean concentration of positive samples was 1.76 µg.kg^{-1}.

Table 2. OTA content on of pure Arabica coffee brand

Arabica Coffee Brand code	Origin	OTA content in ground roasted coffee (µg.kg^{-1})
1	Foreign	4.8± 0.4
2	Foreign	nd
3	Foreign	1.8 ± 0 .5
4	Foreign	1.3 ± 0.1
5	Foreign	0.3 ± 0.9
6	Foreign	nd
7	Foreign	nd
8	Foreign	0.8 ± 0.6
9	Foreign	0.6 ± 0.6
10	Foreign	nd
11	Local	2.3 ± 0.2
12	Local	1.1 ± 0.3
13	Local	0.8 ± 0.1
14	Local	4.9 ± 1.1
15	Local	nd
16	Local	0.7 ± 0.0
17	Local	nd

Pure Robusta coffee samples had much larger OTA content (Table 3): the values found were between <LOD to 18 µg.kg^{-1}. Seventy five percent of samples showed contamination by OTA (values above the LOD of the

method), and fifty percent of positive samples had OTA levels above the limit set.

Table 3. OTA content of pure Robusta brand

Robusta Coffee brand code	Origin	OTA content in ground roasted coffee (µg.kg^{-1})
18	Foreign	1.3 ± 0.9
19	Foreign	0.8 ± 1.0
20	Foreign	nd
21	Foreign	6.3 ± 0.7
22	Foreign	0.8 ± 0.8
23	Foreign	nd
24	Foreign	5.7 ± 0.3
25	Foreign	nd
26	Foreign	11.8 ± 1.0
27	Local	3.7 ± 0.8
28	Local	18.0 ± 1.1
29	Local	1.2 ± 0.2
30	Local	2.3 ± 0.3
31	Local	nd
32	Local	15.1 ± 0.9
33	Local	7.8 ± 1.4

Samples of Arabica and Robusta mixture coffee show 62% of OTA contamination (Table 4). Levels are between the limit of detection of the method and 7.4 µg.kg^{-1}. Twenty percent of positive samples had OTA levels above the limit set.

Table 4. OTA content of Arabica/Robusta mixture coffee brand

Mixture coffee brand code	Origin	OTA content in ground roasted coffee (µg.kg^{-1})
34	Foreign	1.2 ± 0.6
35	Foreign	0.8 ± 0.0
36	Foreign	nd
37	Foreign	nd
38	Foreign	0.9 ± 0.5
39	Foreign	0.7 ± 0.2
40	Foreign	nd
41	Foreign	0.3 ± 0.1
42	Foreign	5.7 ± 0.2
43	Local	nd
44	Local	1.8 ± 1.0
45	Local	2.1 ± 0.8
46	Local	nd
47	Local	7.4 ± 0.1
48	Local	0.6 ± 0.2
49	Local	nd

The dry processing (Robusta coffee) showed significant contamination by OTA. The level of contamination showed significant variation depending on country of origin. This difference can be attributed to climatic conditions, post-harvest treatments, or roasting conditions used by roasters[29, 16, 3, 4, 27].

Similar studies were made in many others countries[30, 2, 7]. Van der Stegen et al.[30] screened OTA levels in different kinds of coffee in eight European countries (Belgium, Finland, France, Germany, Italy, the Netherlands, Switzerland and the United Kingdom), and OTA levels in roasted coffee ranged from0.5 to 8.2 ng/g. The European Commission[10] studied the dietary intake of OTA through several foodstuffs by the population of Denmark, Finland, France, Germany, Norway, Sweden, the Netherlands, The United Kingdom, Italy, Greece, Spain, and Portugal. Each country provided food contamination and consumption data. OTA levels in roasted coffee reached 13.1ng/g. However, in the other studies done by individual countries (Brazil, Canada, Denmark,Germany, Hungary, Japan, Spain, United Kingdom, United States), OTA occurrence in commercial roasted coffee was different for each case and varied from 7 to 100%, whereas OTA levels varied from 0.1 to 17 ng/g, as showed in the compilation of Codex Alimentarius Commission[5]. Thus, our results concerning coffee contamination remain within the ranges of contamination detected in Spain and in other countries.

3.3. OTA Occurrence in Different Ground Coffee Brands

99 out of 147 samples of forty nine different brands presented OTA concentrations above the LOD of the method (<0.03 µg.kg^{-1}). Positive samples ranged from 0.3 to 4.9 µg.kg^{-1} with Arabica coffee brand, 0.8 to 18.0 µg.kg^{-1} with Robusta coffee brand and 0.3 to 7.4 µg.kg^{-1} with coffee mixture brand. Robusta Coffee brands are the most contaminated, follow by coffee mixture brands, and Arabica coffee brands. Different OTA values that surpassed the limit set by the European Union (5 ppm) are respectively 6 of 16 Robusta brands, 2 of 16 mixture brands, and 0 of 17 Arabica brands. Arabica coffee brands are the less contaminated by OTA

For the same type of coffee (Arabica or mixture), no significant differences were found among brands neither among coffee origins (local or foreign). Concerning Robusta coffee, OTA level depend to coffee origin: Robusta coffee from Cameroon is more contaminated than Robusta coffee of foreign origin. Average concentrations are respectively equal to 9.6 and 6.3 µg.kg^{-1} (Table 5).

Table 5. Occurrence of OTA in different coffee brands

Matrix	Brand	Samples	Positive samples	Positive concentration range (µg.kg^{-1})	Mean concentration (µg.kg^{-1})
Pure Arabica coffee	Local	21	15	0.7 – 4.9	2.8
	Foreign	30	18	0.3 – 4.8	2.5
Pure Robusta coffee	Local	21	18	1.2 – 18.0	9.6
	Foreign	27	18	0.8 – 11.8	6.3
Arabica/ Robusta Mixture	Local	21	12	0.6 – 7.4	4.0
	Foreign	27	18	0.3 – 5.7	3.0

3.4. OTA Content in Coffee Drink

In general, an increase in OTA content in beverages is observed compared to that of roasted and ground coffee (Table 6). The average extraction rate of 107% was obtained with samples that are contaminated with OTA (content above LOD).

Table 6. OTA content in coffee drink

Coffee brand code	OTA content in roasted and ground coffee	OTA content in coffee drink	OTA extraction rate
1	4.8± 0.4	2.1± 0.5	43.7%
2	nd	nd	/
3	1.8 ± 0 .5	3.6 ± 0.2	200.0%
4	1.3 ± 0.1	1.8 ± 0.4	138.4%
5	0.3 ± 0.9	nd	/
6	nd	nd	/
7	nd	nd	/
8	0.8 ± 0.6	1.2 ± 0.0	150.0%
9	0.6 ± 0.6	nd	/
10	nd	nd	/
11	2.3 ± 0.2	3.9 ± 0.9	169.5%
12	1.1 ± 0.3	0.6 ± 0.2	54.5%
13	0.8 ± 0.1	nd	/
14	4.9 ± 1.1	3.3 ± 1.1	67.3%
15	nd	nd	/
16	0.7 ± 0.0	nd	/
17	nd	nd	/
18	1.3 ± 0.9	0.9 ± 0.3	69.2%
19	0.8 ± 1.0	nd	/
20	nd	nd	/
21	6.3 ± 0.7	5.9 ± 0.9	93.6%
22	0.8 ± 0.8	nd	/
23	nd	nd	/
24	5.7 ± 0.3	6.3 ± 0.6	110.5%
25	nd	nd	/
26	11.8 ± 1.0	13.0 ± 1.1	110.1%
27	3.7 ± 0.8	2.3 ± 0.5	62.1%
28	18.0 ± 1.1	20.1 ± 0.7	111.6%
29	1.2 ± 0.2	3.2 ± 0.0	266.6%
30	2.3 ± 0.3	1.4 ± 0.1	60,8%
31	nd	nd	/
32	15.1 ± 0.9	16.7 ± 0.8	110.5%
33	7.8 ± 1.4	8.0 ± 0.7	102.5%
34	1.2 ± 0.6	1.1 ± 0.6	91.6%
35	0.8 ± 0.0	nd	/
36	nd	nd	/
37	nd	nd	/
38	0.9 ± 0.5	0.5 ± 0.0	55,5%
39	0.7 ± 0.2	nd	/
40	nd	nd	/
41	0.3 ± 0.1	nd	/
42	2.7 ± 0.2	1.0 ± 0.3	37.0%
43	nd	nd	/
44	1.8 ± 1.0	1.3 ± 0.0	72,2%
45	2.1 ± 0.8	3.0 ± 0.7	142.8%
46	nd	nd	/
47	2.8 ± 0.1	3.9 ± 1.2	139.2%
48	0.6 ± 0.2	nd	/
49	nd	nd	/

These results are similar to those found by many scientists who observe an extraction rate up to 132% in the coffee beverage[28, 27, 29].

Considering the fact that the official method for OTA determination in roasted coffee recommends extraction at room temperature and the drink is hot-prepared, there is a problem of OTA extraction in roasted coffee.

To better understand this phenomenon, Mounjouenpou et al.[20] studied the extraction of OTA in roasted coffee, depending on the temperature and time of extraction. It came out that the optimum couple time - temperature for the OTA extraction is 50min - 60°C. Improved extraction rate up to 300% is observed.

3.5. Assessment of the Exposure to OTA by through Coffee

The average OTA contamination of coffee drinks from different brands are 0.73µg.kg^{-1} for pure Arabica coffee brands, 4.61µg.kg^{-1} for pure Robusta coffee brands and 0.57µg.kg^{-1} for Arabica / Robusta mixtures brands.

Cameroonian coffee consumption is estimated at 1ml/day (source FAO/Cameroon).

Given the average body weight of an adult who is 70 kg and maximum OTA contamination level obtained with coffee beverage brands from pure Robusta coffee (4.61 µg.kg^{-1}), the daily intake of OTA is 0.065 ng / kg bw per day. This value represents 1.3% of the TDI (Tolerable Daily Intake) defined by 'the Scientific Committee on Food of the European Commission (5ng/kg bw per day) and 0.4% of' "WHO Committee of Experts on Food Additives (14 ng/ kg bw per day) (FAO/WHO, 2001; Commission of the European Communities, Directorate General XXIV, 1998).

Consumption of OTA from coffee in the cities of Douala and Yaoundé are very low compared to that of Europe who is 2-3 ng/kg bw per day[11, 25].

Consumption of OTA from coffee in the cities of Douala and Yaounde is very low compared to that of Europe, for which the value is 2-3 ng/kg bw per day[11, 25]. Robusta coffee consumers are more exposed than other consumers.

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