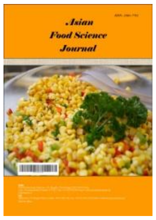

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Aspergillus Producing-Ochratoxin A During Coffee Processing in Cameroon: A Short Literature Review and Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was aimed at determining the distribution of ochratoxin A (OTA) in different types of coffee using short review and analysis. Mycotoxins have undoubtedly presented a global challenge to human health since the earliest times, and this threat will mainly increase as the demand for the available food supply increases in response to the growth of the world population. The most important naturally occurring mycotoxins in human food are aflatoxin, ochratoxin, deoxynivalenol, zearalenone and fumonisin. OTA is the most prevalent and toxic and is produced by both *Aspergillus* and *Penicillium.* Coffee production has high socio-economic importance in most African countries. However, postharvest treatment and storage conditions represent optimal environments for the occurrence of fungi responsible for mycotoxins production in coffee beans. Preliminary data revealed the wild diversity of coffee beans with the presence of other fungal genera such as *Mucor*, *Cladosporium, Fusarium, Wallemia* and *Acremonium.* In addition, the authors found the production ability of *A. niger*, *A. carbonarius* and *A. ochraceus* to produce

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Ochratoxin-A at concentrations of 0.31, 5.07 and 13.08 µg.kg^{-1}. The outcome of this short review can be used for the building of a risk evaluation procedure aiming to provide specific actions to reduce the exposure to OTA in coffee beans and to improve the exchange of coffee products throughout the world.

Keywords: OTA; aspergillus diversity; coffee; cameroon.

1. GENERALITIES ON COFFEE

Coffee is one of the raw materials mostly exchanged throughout the world which occupies an important place in terms of amount and value in the culture of agricultural rent products [1]. This sector provides employment of about 100 million people, and 25 million are involved in cultivation and harvesting [2]. On average, the world harvest is about 8.9 M tons (2015 – 2016) (Table 1). South American countries provide almost half of this amount and the largest producers are Brazil, followed by Vietnam and Colombia [3]. In Africa, Cameroon is among the countries having an important part in the coffee market [3]. Even if the coffee branch is rapidly increasing, some problems can be noticed in its development, such as a fall in world prices, a fall of incomes, low production yields and difficulties of farmers in appropriating novel or recent production techniques. The consumption of coffee per capita has been constant for the last two decades and, industrial countries consume about 75% of world coffee [1,3]. Moreover, coffee has a great potential quality which strongly depends on the tree variety, cultural conditions, seasons, geographic origin, and agronomic knowledge of farmers. This potential can be improved or lost during the postharvest operations [4,5]. The processing technology of coffee is too long (as described in Fig. 1) and requires a primary process of handling. In addition, there is treatment through dry and/or wet routes as well as storage and transportation [6,7]. During postharvest treatments, fermentation participates in the development of the organoleptic properties and merchantable qualities of the products obtained [8]. Therefore, the absence postharvest treatment of coffee beans can lead to the apparition of some microbial activities with a negative effect on the health status of the consumer and significant economic losses [2]. Fungi producing mycotoxins are the main microbial group incriminated. The European Union is the major destination for coffee produced in Cameroon and OTA coupled with aflatoxin B1 (AFB1), are the most monitored and controlled mycotoxins.

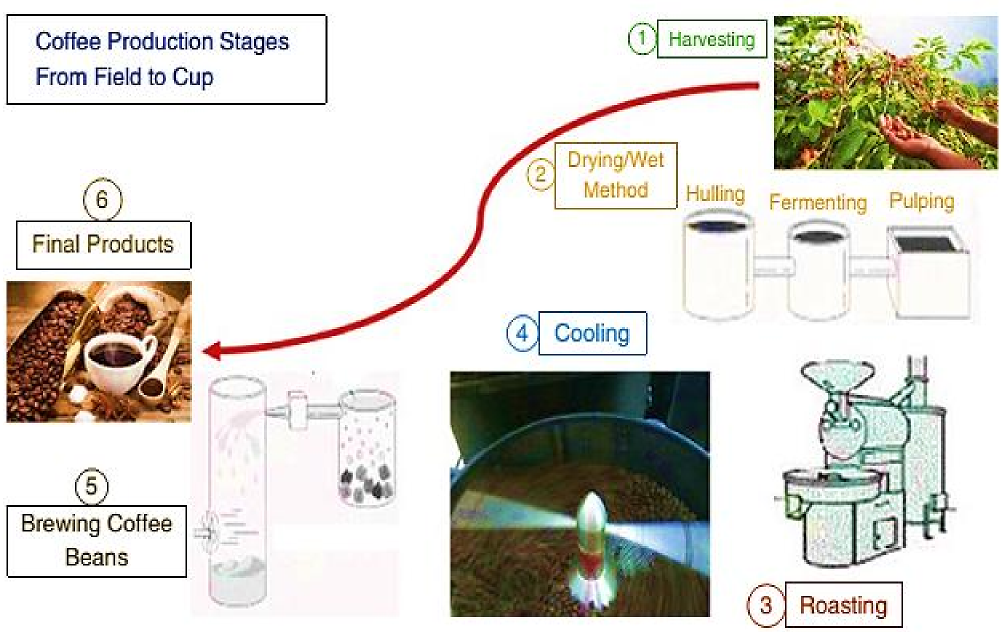

Fig. 1. Diagram showing stages of coffee [9]

Table 1. The global production of coffee

Crop year	2013–2014	2014–2015	2015–2016
Coffee variety			
Arabica	90 540*	85 239	85 814
Robusta	61 564	61 410	62 179
World total	152 105	146 648	147 994
Africa			
Ethiopia	6527	6625	6700
Uganda	3633	3744	4000
Ivory Coast	2107	1750	1900
Tanzania	809	728	875
Kenya	838	675	760
Cameroon	404	483	570
Madagascar	584	500	449
Congo, DR	347	335	335
Rwanda	258	238	278
Burundi	163	248	274
Togo	135	185	200
Guinea	101	147	177
Others	299	255	312
Total	16 205	16 005	16 831
Asia & Oceania			
Vietnam	27 610	26 500	27 500
Indonesia	10 300	9 935	11 525
India	5 075	5 450	5 833
Papua New Guinea	835	798	712
Philippines	593	603	605
Laos	550	506	520
Thailand	638	497	500
Yemen	185	150	130
Others	117	154	102
Total	45 903	44 592	47 428
Mexico & Central America			
Honduras	4 568	5 400	5 400
Guatemala	3 439	3 310	3 400
Mexico	3916	3 591	2 800
Nicaragua	1 941	1 898	2 100
Costa Rica	1 444	1 408	1 492
El Salvador	525	698	565
Dominican Republic	425	397	400
Haiti	345	343	342
Panama	113	106	107
Cuba	107	101	100
Others	32	34	33
Total	16 856	17 287	16 739
South America			
Brazil	55 050	51 116	48 423
Colombia	12 124	13 333	14 009
Peru	4 338	2 883	3 301
Ecuador	666	644	644
Venezuela	805	651	500
Bolivia	128	106	89
Others	30	31	30
Total	73 141	68 764	66 997

** In thousand 60 kg bags; Source: Adapted from ICO [3] (2016)*

2. OCHRATOXINS (OTA) IN COFFEE

2.1 Legislation

OTA (Illustrated in Fig. 2) is classified in Group 2B by the International Agency for Research on Cancer as a possible human carcinogen, which has raised important concerns about human exposure to this mycotoxin [10]. In addition, OTA absence is one indicator of the safety criteria required for international trade of co□ee because of its deleterious effects on humans' health status, such as teratogenic, immunotoxic, nephrotoxic and carcinogenic effects [10,11,12]. Therefore, the limits tolerable for OTA in foodstuffs, especially for food products with a higher risk of OTA occurrence, including coffee beans, were established by several countries [10,13]. This mycotoxin is naturally produced by several species belonging to the *Penicillium* and *Aspergillus* genera. Moreover, researchers reported that among these microorganisms, *Aspergillus* species such as *Aspergillus carbonarius, A. niger* and *A. ochraceus* are the most widely OTA producers in tropical and semi-tropical coffee plantations, while *Penicillium* species such *as P. verruculosum, P. brevicompactum, P. crustosum, P. olsonii and P. oxalicum* are usually found in temperate regions [7,14,15].

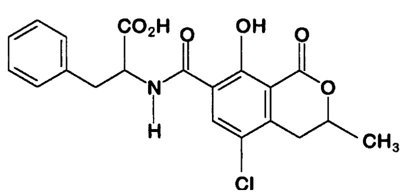

Fig. 2. Chemical structure of OTA

These fungi species are able to grow in different abiotic conditions, on different plants, and so contamination of several food crops with OTA can occur in the world. These plants or human foods include meat products as a result of contamination of animal feed, cereals, dried vine fruit, beer, wine, cocoa, as well as coffee. OTA is toxic to animals and the principal effect being reported is nephrotoxicity. The human health risks associated to OTA have been evaluated and discussed at both European and international levels by the European Commission Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), who have established acceptable intakes of OTA from several foods [13,16,17]. These tolerable values have been used in several countries worldwide as a basis for regulation of OTA in some foods. EFSA adopted on April 4, 2006, at the request of the European Commission, that the tolerable weekly intake of OTA is 0.12 µg/kg body weight [9]. The main contributors to OTA exposure are cereals and cereal products. Wine, coffee and beer are also important contributors to human exposure to OTA. Dried vine fruits and grape juice also contribute to a significant portion of OTA exposure to certain vulnerable consumer groups, such as children. The OTA content of coffee as reported for some products (Table 2) depends mainly on the fungal flora present as well as on the OTA content of coffee beans, a raw material for which OTA content has not been sufficiently studied scientifically in recent decades [9]. Thus, the appropriateness of setting a maximum level for OTA or revising existing maximum levels in coffee, particularly in coffee beans, should be considered in the light of recent scientific data.

Several studies have elucidated the relationship between frequency, toxigenic potential of mold species belonging to previous genera and the postharvest treatments of coffee [18]. However, there is not sufficient information concerning the mechanism of contamination by mycotoxin-producing fungi, particularly *Aspergillus* species [19].

2.2 Distribution

Nowadays, the three major species of mould that produce OTA are *Penicillium verrucosum, Aspergillus ochraceus* and *Aspergillus carbonarius*. They mainly differ in physiology and microbiota, which in turn affects the variety of foods in which these moulds are most commonly found. For example, *A. carbonarius* grows optimally at 32–35°C and dried wine fruits such as raisins and wine constitute the major habitat. On the other hand, the major habitat of *P. verrucosum* is cereal crops in the cool and temperate climates of northern Europe and America [10]. Consequently, OTA is mainly found in cereal products such as flour-based foods. It is also found in commodities such as cheese and meat products from animals that eat cereals as a major dietary component. In contrast, *A. ochraceus* is most found in dried and stored foods, such as smoked and salted dried fish, chick peas, nuts, pepper, soya beans and dried fruit. It has been reported infrequently in green coffee beans. *A. ochraceus* is commonly present at low levels and rarely causes spoilage. Its presence may not be a good indicator of significant OTA contamination (JECFA, 2001). Finally, *Aspergillus niger* is often found in association with *A. carbonarius* is commonly isolated from foods in warm climates. Similarly, OTA occurrence in coffee is widely associated with geographical zones and coffee processing.

Table 2. Maximum levels (ng/g) for ochratoxin A in foods [9]

Coffee products	Maximum level
1. Roasted coffee beans and ground roasted coffee, excluding soluble (instant) coffee	5
2. Soluble (instant) coffee	10
3. Green coffee, dried fruit other than dried vine fruit, cocoa and cocoa products	/

Table 3. OTA contents of the different samples of coffee analyzed

Site	Processing technique	Type of coffee	Processing step	OTA (µg/kg)	
				2017	2018
Bafoussam	DP	A	I	4,6	3,1
			III	0,1	nd
		R	I	8,8	1,8
			III	0,6	nd
	WP	A	II	0,7	1,3
			III	nd	nd
Dschang	DP	A	I	4,1	0,6
			III	nd	nd
		R	I	7,3	4,8
			III	0,1	0,8
	WP	A	II	1,6	0,8
			III	nd	0,3
Bafia	DP	R	I	7,9	4,7
			III	0,7	0,3
Batouri	DP	R	I	12,7	3,3
			III	0,7	0,5

DP: dry process; WP: wet process; A: arabica; R: robusta; I: dry cherries; II: dry parchment; III: green coffee beans; n.d.: not determined

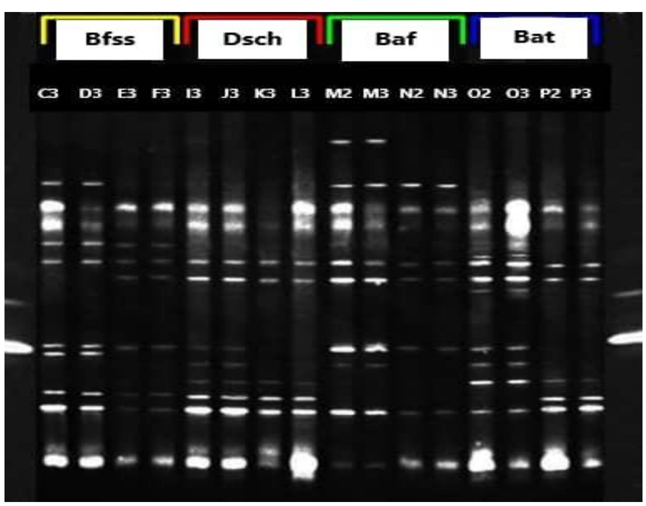

Fig. 3. DGGE profiles of PCR amplicons of the domain D1 of 28S rDNA that represent the fungi biodiversity in of coffee. Markers (1, 2); Bfss: Bafoussam; Dsch: Dschang; Baf: Bafia; Bat: Batouri. The position of bands is indicated by numbers that correspond to species of fungi [20]

2.3 Geographical Distribution of OTA in Coffee

Ochratoxin A (OTA) contamination is commonly associated with several food matrices such as cereals, dried fruits, wine, cocoa, and coffee. Authors mentioned that cereals and derived products (more than 50%) are a major source of human OTA exposure, while wine and coffee take second and third place with a contribution of about 13% and 10% respectively. [17]. OTA has been found in coffee sourced from all over the world [21]. The presence of OTA in coffee was preliminary reported by Levi [22] and in other coffee products such as green coffee beans [23, 24], roasted coffee [22], and instant coffee [25, 26]. In addition, Pérez de Obanos et *al*. [27] detected the presence of OTA in brewed coffee. The main OTA-producing species in coffee beans belong to the genera *Aspergillus* Section *Circumdati* and Section *Nigri* [28, 29]. Many *Aspergillus* species produce OTA in coffee such as *A. ochraceus* [29-33], *A. niger* [30, 32], *A. carbonarius* [30, 33, 34], *A. sulphureus* [31], *A. sclerotiorum* [31], and *A. westerdijkiae* [31, 32], but the most studied species are *Aspergillus niger, A. carbonarius* and *A. ochraceus* [14]. These three species have been identified in 408 coffee samples from four regions of Brazil and their distribution depended on stage of maturation and geographic site [30]. From all samples analyzed, 872 isolates of *A. ochraceus*, *A. niger*, and *A. carbonarius* were obtained. *A. niger* was the most prevalent species (63%) and 3% of them produced OTA. *A. ochraceus* and *A. carbonarius* were also identified with a prevalence of 31 and 6% respectively. About 75% of *A. ochraceus* and 77% *A. carbonarius* isolated produced OTA. This observation showed that OTA production capacity is strain, time and matrix-dependent. According to variation in geographical site, researchers reported that OTA detection in coffee beans is more prevalent in Thailand than in Brazil [34, 30]. Moreover, Alvindia and Guzman [15] in Philippine mentioned that *A. niger* was the dominant species compared to *A. ochraceus* and genus *Penicillium* had a 16% share in the total fungal flora of coffee beans. In Cameroon, data from coffee samples collected from different geographical zones illustrated the fungal diversity with the geographical zone characterized by the number and position of bands (Fig. 3) [20]. OTA detection tests on the same collected samples revealed that geographic site and period significantly influenced the OTA content of coffee samples (Table 3). This study also mentioned that variation in environmental and storage conditions significantly contributed to increasing the level of OTA. Sequencing of gene-encoding 28S RNA revealed the occurrence of several fungal species belonging to the genera *Aspergillus, Penicillium, Mucor*, *Cladosporium, Fusarium, Wallemia*… Among the *Aspergillus* genus, *A. fumigatis*, *A. carbonarius, A. ochraceus and A. niger* were identified and OTA quantification tests showed that *A. ochraceus* was the most producer followed by *A. cabonarius* and *A. niger* with an average production of 13.08, 5.07 and 0.31 $\mu g.kg^{-1}$ respectively after 20 days.

3. CONCLUSION

The risk that ochratoxin A (OTA) generates for consumer health has led the sanitary authorities of the European Union to establish maximum allowable limits for several agricultural commodities, particularly coffee. Real-time PCR and conventional PCR have been used in research laboratories to detect ochratoxin A producing by *Aspergillus* in coffee samples, to obtain information on the epidemiology and ecology of ochratoxigenic species or to acquire basic information on gene expression. Simplicity, sensitivity, and specificity of analysis are remaining challenges. Furthermore, OTA biosynthesis is poorly understood relative to the synthesis pathways of other economically important mycotoxins.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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