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Ochratoxin A in coffee. A critical review of contemporary scientific literature

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Abstract– ***Postharvest activities and bean storage conditions play an important role in preventing fungal infestations in coffee. Little can be done to prevent coffee fruit from being contaminated in the field with filamentous and saprophytic fungi responsible for the ochratoxin A biosynthesis; However, good postharvest practices coupled with short storage periods can reduce the likelihood of fungal infestation. Fortunately, ochratoxin A is a secondary metabolite which means it is not essential for the fungus survival, which is why the fungi presence does not necessarily cause grain contamination with the mycotoxin.***

However, even when the coffee drink is not considered an important source of the mycotoxin due to its thermal degradation during the grain roasting, its water solubility added to the coffee drink daily consumption during long periods of time can result in the development of hepatic and renal pathologies, as demonstrated through animal models.

The following discussion is the product of an extensive review of current scientific literature which aims to describe the most salient topics associated with ochratoxin A and coffee, its metabolism, its toxicity, consumption limits, solubility, extraction, as well as a discussion on the analytical techniques used for its determination.

Keywords- mycotoxin, synthesis, extraction, ELISA.

I. INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin belonging to the isocoumarins which was isolated for the first time in 1965 and later discovered in maize samples in 1969 [1,2], but its presence in coffee beans samples was reported for the first time by Levi, Trenk & Mohr in 1974 [2-4]. However, until recently it was thought that OTA was degraded during the grain roasting, however its presence in roasted coffee and in the coffee beverage was reported by Tsubouchi, Yamamoto, Hisada, Sakabe & Udagawa in 1987 [2,4].

Mycotoxins are secondary metabolites of fungal origin with pharmacological activity that can cause serious health problems or even death to humans or animals when ingested, inhaled, or absorbed. At room temperature, OTA appears, under the visible light spectrum, as colourless crystals, and it also has no commercial value therefore is not industrially produced [5].

OTA shows a molecular structure that consists of a *para*-chlorophenolic fraction which contains a 3,4-dihydro-methyl-iso-coumaric ring, linked through its carboxyl group through an amide type bond to a L-β-phenylalanine molecule (Fig. 1) [1,3]. OTA is an organic, aromatic, heteropolycyclic and thermostable compound, which is very unstable under the spectrum of visible light; However, it remains stable in dark EtOH-based solutions [5].

OTA is synthesized by filamentous and saprophytic fungi, which are neither phytopathogenic nor endophytic, so their presence in coffee fruits is due to their opportunistic nature [6]. In contrast, an endophytic entity is an organism, often a bacterium or fungus, that lives within a plant, in a symbiotic relationship, for at least a fraction of its life cycle without causing disease.

Specifically, the fungi responsible for the production of OTA in coffee fruits are two sections of the genus *Aspergillus*: *Nigri* (black) (e.g., *A. niger* van Tieghem & *A. carbonarius*) and *Circumdati* (yellow) (e.g., *A. ochraceus* Wilhelm , *A. sulphureus*, *A. westerdijkiae*, & *A. sclerotiorum*) [7,8]; Likewise, it is also produced by *Petromyces alliaceus*, *Neopetromyces muricatus* and species of the genus *Penicillium* (e.g., *P. viridicatum*, *P. verrucosum* & *P. nordicum*) [4,6,9-14].

OTA-producing species of the genus *Penicillium* are usually associated with growing areas with temperate climates and display lower mycotoxin production [5,13,15]. In contrast, OTA-producing species of the genus *Aspergillus* (especially *A. ochraceus* & *A. westerdijkiae*) are often found in growing areas with warmer climates [4,5,13,15]. Likewise, there are areas of the world that present a greater or lesser incidence of the problem; However, no coffee-producing country is free of such contamination.

On the other hand, *A. ochraceus* is the most important OTA-producing species but it is often confused with *A. westerdijkiae* because they are morphologically very similar [13,16]. Another example of confused identity is the case of *A. carbonarius* and *A. ibericus*, two taxonomically very close species that show similar morphological characteristics [16]; However, *A. carbonarius* is an OTA-producing species while *A. ibericus* is not, which is why many results have been reported in which *A. carbonarius* is presented with a variable OTA production capacity [16].

Nowadays, due to phenotypic similarities between different species, it is more appropriate to differentiate them through molecular studies, such as genes sequence analysis as in the case of β-tubulin or calmodulin [16].

Curiously, the evidence suggests that the presence of OTA-producing mycopathogens does not necessarily translate into high levels of OTA in infected samples [12,13] due to the fact that the mycotoxins production is not an essential function

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for the pathogen survival. For this reason, if OTA-producing fungi are present, a chromatographic analysis is required to confirm the mycotoxin's presence.

II. OTA SOURCES

Coffee is the source of 6-9% of the total OTA consumed by humans, being surpassed by wine (10%) and cereals (44%) [9,15,17]. For instance, in the United States of America, oat derived products are responsible for 30 & 70% of OTA exposures among the adult and infant population, respectively [8].

However, OTA can also be found in a number of food products such as nuts, grapes, pork and poultry, beer [1,4,6,15,18], corn, grapes, grape juice, cocoa, chocolate [1,4,5,8], peanuts, stored grains, cottonseed, bread, flour, rice, peas and beans among others [1,4,5]. While OTA concentrations are higher in red wines than in rosé wines; In beer, the process to which the barley grain is subjected practically eliminates the presence of the mycotoxin from the final product, being present only in those cases when the grain is highly contaminated [5].

Interestingly, evaluations conducted in Europe suggest that coffee bean batches from Central and South America have a lower incidence of OTA regardless of the type of processing applied to them (wet vs. dry) [18].

III. METABOLISM

OTA is water soluble, so almost 100% of it passes into the coffee drink during its preparation; and the concentration variations in the infusion are caused by the drink's method of preparation and the amount of coffee used during its preparation [19].

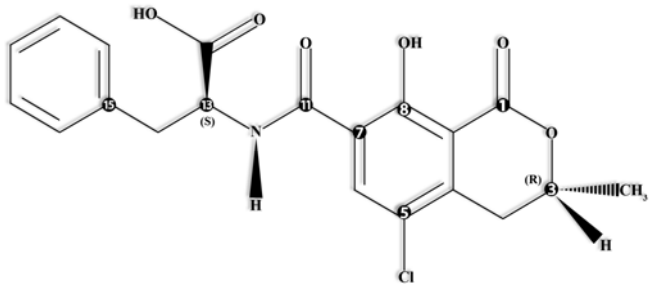
OTA is absorbed through the stomach and small intestinal mucosa; and once in the bloodstream, it adheres to plasma proteins (e.g., albumins) [20] thus becoming a mobile reserve of OTA which has a half-life of 35 days [21,22] before being excreted. Depending on the levels present in the blood, it can be stored in the tissues (e.g., kidneys, liver, mammary glands, muscles and fatty deposits) or excreted through breast milk, constituting a risk for the newborn, through urine at a rate of 20-80 ng day^{-1} [8,10,23] or through the sweat and feces.

During its metabolism, OTA is conjugated with glutathione (GHS), thus producing its activation into a genotoxic and carcinogenic compound. Subsequently, after its activation, both OTA and the metabolites derived from its thermal degradation (e.g., 2'R-ochratoxin A) (Fig. 1) exert their genotoxic (mutagenic) action by coupling to guanine and adenine molecules through covalent bonds thus producing DNA adducts [21,23,24] which is considered the initial stage in the cancers development.

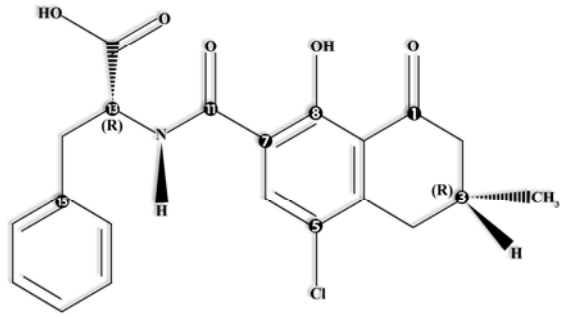
IV. TOXICITY

OTA is the most studied mycotoxin in coffee and is considered a hepatic and nephrotoxin that affects birds and mammals, so its ingestion can cause liver histological abnormalities and promote the development of endemic nephropathy in the Balkans, a kidney disease characterized by the development of tumours in the urinary tract (urothelial tumours) [10,14,23].

OTA is a secondary metabolite that has a teratogenic effect (A teratogenic agent is a substance, physical agent, or organism capable of causing congenital defects during the fetus gestation) since it crosses the placenta and accumulates in the fetus' tissues causing malformations especially at the level of the central nervous system [6,14,15,23,25] since this has an affinity for the brain and more specifically for the cerebellum, ventral midbrain and hippocampal structures [26].



Ochratoxin A
N-[(5-Cloro-3,4-dihidro-8-hidroxi-3-metil-1-oxo-1H-2-benzopiran-7-il)carbonil]fenilalanina.
CAS: 303-47-9.
Molecular formula: $C_{20}H_{18}ClNO_6$
Molecular mass: 403.818 g mol^{-1}



2'R-Ochratoxin A (2'R-OTA)

Fig. 1. Structure of Ochratoxin A and its thermic derivative.
Adapted from Reference [21].

Although there is sufficient evidence of OTA's carcinogenic effect derived from animals tests (rats) [21] in which it tends to induce adducts formation with DNA in the kidneys of test animals [8]; There are not enough confirmations of the same effect in humans, which is why the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) classified it as "Class 2B", in other words, a possible human carcinogen [5,8,10,12,23].

On the other hand, evidence suggests that cattle are resistant to OTA-contaminated grain; In contrast, in poultry (>

2 μg g^{-1}) it causes a reduction in their growth rate, as well as in oviposition. Likewise, in pigs the consumption of OTA contaminated food causes the development of porcine nephropathy [15].

Finally, since OTA contains a phenylalanine molecule in its molecular structure, it behaves as an antagonist of it, competing with the amino acid in various reactions in which it is required, such as the reaction mediated by the phenylalanyl-tRNA enzyme synthetase thus preventing protein biosynthesis [27].

V. Consumption Limits

Based on the results obtained with pigs, the food additives and contaminants committee integrated by the Food and Agriculture Organization (FAO) and the WHO established a maximum permissible limit for OTA consumption of 100 ng kg^{-1} of live weight/week, which is equivalent to ~ 14 ng kg^{-1} of live weight/day [8,18,28], since at this concentration its excretion, through urine, is 10 to 50 times faster [15,23].

However, the Scientific Committee on Food of the European Commission and the IARC reduced this limit to 5 ng kg^{-1} of live weight/day [8,9,18,19] while the Ministry of Health of Canada established a maximum permissible intake of 4 ng kg^{-1} live weight/day [8]. Unfortunately, the current global weekly OTA consumption is approximately 8 to 17 ng kg^{-1} live weight [8].

Although, at present, there are no regulations regarding maximum permissible limits of OTA in raw coffee beans, in 2005 the European Commission established limits for OTA's concentration in samples of roasted and soluble coffee of 5 and 10 μg kg^{-1} of dry matter respectively [4,13,24,29,30]. However, some countries have established their own regulations regarding maximum permissible limits in coffee, such is the case of Italy with a maximum limit of 8 and 4 μg kg^{-1} of dry matter for raw coffee beans and derived products respectively; 5.0 μg kg^{-1} in Finland [4], 20 μg kg^{-1} of dry matter in Greece, 5 μg kg^{-1} in Switzerland [4,18] and 10 μg kg^{-1} of dry matter for roasted coffee and soluble coffees in Brazil [7,27]. With all this, OTA has become the only coffee mycotoxin for which there is international legislation.

However, the maximum permissible concentration in the raw and dispersed grains is 20 ppb, which is why concentrations higher than this can harm the coffee grain commercialization [13,30] especially in the international market to the detriment of the economies of coffee-producing countries.

VI. Determination of OTA in Coffee Beans

The presence of OTA in coffee beans can occur due to environmental contamination (e.g., storage and transport period) [13,19,30], in addition to the processing method (e.g., wet, dry or mechanized milling) [12].

However, once OTA is present, it is easily detected (when the coffee beans are incubated in a culture medium based on agar and cocoa cream) thanks to its typical blue and green fluorescence when exposed to u.v. light [5,6,19,29] at 230-336 nm [14,31].

Mycoflora analyses reveal the presence of the species that infest coffee beans; However, although species of the *Aspergillus* genus can produce high levels of OTA when grown in artificial growth media, this does not mean that the same is occurring in the stored grains from which the analysed samples were extracted [29].

The methods used for OTA's determination in coffee samples require the metabolite's previous extraction in aqueous solution as well as an adequate cleaning process to eliminate other compounds (e.g., lipids, & pigments) that could interfere with the chromatographic analysis in the form of unidentified peaks in the chromatogram [17].

However, the evidence suggests that OTA concentration in solution varies depending on the extraction method [24]. In experiences documented by Reference [20], a variation in OTA concentration in solution was recorded when using three different extraction methods (Table 1). For this reason, attention should be paid to the extraction method used prior to the determination of the OTA's absolute concentration in roasted coffee samples [20].

The enzyme-linked immunosorbent assay (aka ELISA) is a rapid, inexpensive laboratory technique, which does not require highly specialized technicians, and is commonly used to measure the OTA concentration in solution based on the use of monoclonal and polyclonal antibodies inoculated in animals [1,24]. In addition, it is a useful technique for the rapid analysis of samples from different matrices, which is why it has become a routine test to monitor the presence of mycotoxins in food matrices.

For the development of the analyses, different kits can be found on the market, with the necessary reagents for the analysis, and with methods that have been developed and optimized by the companies that market them [32].

Table 1. Effect of the extraction method on the OTA concentrations in solution.

Roasted and ground coffee		Extraction method		
		Filtered coffee	French coffee	Express coffee
OTA (μg kg^{-1}).	24.7 ± 0.1	30.1 ± 0.1	30.1 ± 1.7	18.4 ± 0.7
Recovery (%).	100	122	122	75

Adapted from Reference [20].

The method is based on the ability of a specific antibody to distinguish the three-dimensional structure of a given mycotoxin (antigen-antibody reaction). Mycotoxins are extracted from samples with organic solvents (e.g., acetronitrile, water, or MeOH plus sodium bicarbonate) followed by dilution in a buffered saline (e.g., MeOH-phosphate) [4,33].

To carry out the analysis, the following reagents are mixed and added to the microtiter wells coated with specific

antibodies for the analysed mycotoxin: a portion of the recovered extract, a portion of a mycotoxin-enzyme conjugate, and a portion of anti-mycotoxin antibodies mycotoxin.

The system is incubated, at room temperature (20 ºC) and in a controlled light environment for 30 min to 2 hours, allowing free mycotoxins present in the sample or standard to compete for binding to specific sites of the anti-mycotoxin antibody immobilized on the plate (Fig. 2.b, 2.c), which is why this technique is called a competitive enzyme immunoassay.

After incubation, a wash is carried out with distilled water or with saline buffer, to eliminate the substances that did not bind to the system in a specific way (Fig. 2.d). Subsequently, a substrate/chromogen [peroxide/tetramethylbenzidine (TBM)] is added, followed by a second incubation where the mycotoxin-enzyme conjugate through the antibodies converts the chromogen into a blue substance (Fig. 2.e).

After a reaction time, a stop solution consisting of dilute sulfuric acid is added, since the acidic environment causes a change from blue to yellow (Fig. 2.f). Finally, the measurement is performed photometrically at 450 nm using an ELISA reader; The absorbance is inversely proportional to the concentration of mycotoxin in the sample [34,35].

Subsequently, the samples' optical densities (OD) are compared with the standards' OD to carry out the calculation corresponding to each mycotoxin and quantitatively determine its concentration. On the other hand, there is other method designed for the OTA analysis in coffee samples reported by Reference [33] which is carried out under the methodology outlined in fig. 3.

However, OTA can also be determined through HPLC-type analysis, liquid chromatography combined with mass spectroscopy [17,24] or radio immunoassays (RIA).

In the chromatographic analysis, OTA is identified by comparing the retention time (Tr) of each metabolite present in the analysed sample vs the Tr of a standard sample of the metabolite of interest and its quantification is achieved through the measurement of emitted fluorescence either through its ability to absorb u.v. light or graphically through the peaks on a chromatogram.

VII. OTA'S PREVENTION AND DEGRADATION IN COFFEE BEANS

Although OTA cannot be completely eliminated from food, the following measures are proposed to reduce its incidence in coffee beans and thus protect the consumer from unnecessary exposure. After all, prevention is better than cure.

7.1 IN THE COUNTRYSIDE

The highest risk of exposure to the fungi responsible for OTA biosynthesis occurs in the field when coffee fruits come into direct contact with the soil as they are knocked over by rain, wind or human activities [3,13] or when these are perforated, at any stage of development, by borer insects (*Hypothenemus hampei*) [2,6,13,36,37] which defecate inside the fruits and carry, attached to their bodies, fungal conidia, thus creating favourable conditions for their development and the subsequent OTA synthesis.

Although the fruits protection in the field is more difficult to achieve due to their exposure to the elements, one of the recommended agronomic practices to prevent the incidence of OTA-producing agents is to grow coffee in temperate climate zones where the environmental conditions are unfavourable for the development of fungal infections [13].

In addition, it is recommended to avoid sprinkler irrigation during the flowering stage to avoid creating favourable conditions for the dissemination of spores producing fungi [36] or to prevent the colonization of phytopathogenic fungi such as *Cercospora caffeicola* Berk & Cooke Hill the causal agent of cercosporiasis [6].

Likewise, the grains that are usually collected from the ground to prevent the reproduction of *Hypothenemus hampei* (pepena) should not be incorporated with the rest of the harvested grains, since they are more likely to have been infected by fungi [6,36] reaching concentrations above the permissible limit (5 μg kg^{-1} of dry matter) [6] especially in the case of black and sour grains (Table 2) [7,13,36] due to a higher incidence of *Aspergillus* species (Section *Nigri*) and *Aspergillus westerdijkiae* [38].

Table 2. OTA (mg kg^{-1}) in healthy and defective Brazilian coffee beans.

Type of defect.	Cerrado, Minas Gerais.	Sorocabana, São Paulo.
Healthy grains.	ND[a]	1.4
Green beans (immature).	ND	0.3
Black beans.	0.5	25.7
Black-green grains.	0.4	0.3
Sour grains.	11.3	0.3

[a] Not detected (detection limit 0.2 μg kg^{-1}). Adapted from Reference [7].

7.2 DURING THE GRAIN PROCESSING

Once the fruit is invaded by mycopathogens, OTA biosynthesis takes place in the pericarp (e.g., epicarp, mesocarp, & endocarp) and is later transferred to the grains. For this reason, the pulping process during wet milling contributes significantly to reducing OTA levels in raw coffee beans [13].

Another important route of exposure occurs during the grain processing when it comes in contact with contaminated surfaces used for drying in the sun [13,24] so it is recommended to do it on concrete or asphalt platforms, avoiding those made directly on the ground even if it has been compacted since this is the natural habitat of these fungi [6,13].

To accelerate the drying of the grain parchment on the drying platform, it should be dispersed producing a thin grain layer with a thickness of no more than 3-5 cm (~ 25-35 kg m^{-2}) which should be stirred several times a day to turn the grain [14,36].

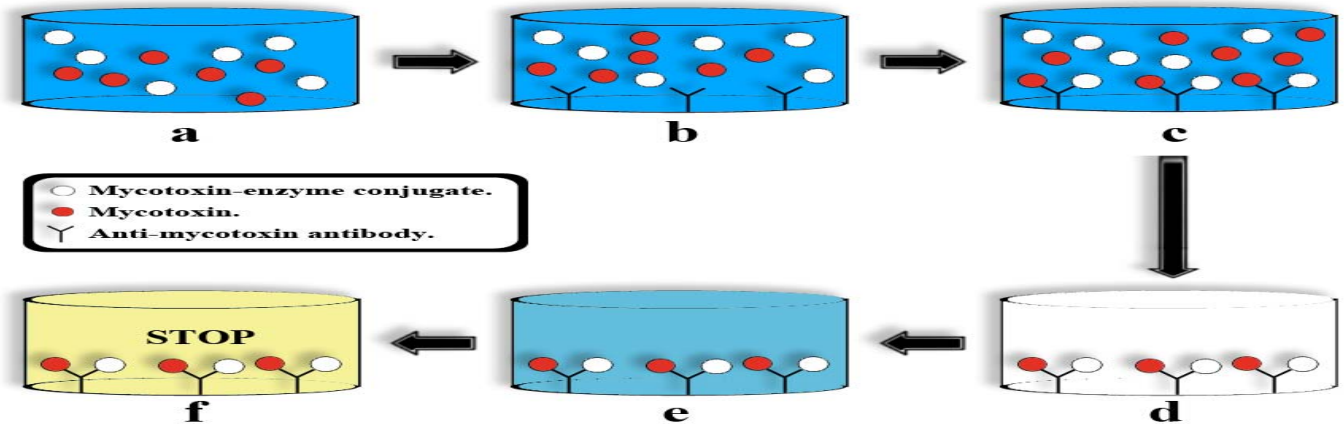


Fig. 2. Scheme of the competitive ELISA method for the quantitative determination of mycotoxins. Adapted from Reference [35].
a) Sample mixed together with the conjugate, b) Mixture poured into microtiter wells coated with antibodies specific for the analyzed mycotoxin, c) The mycotoxin binds to the antibodies in the 1st incubation d) uncoupled materials are removed by washing the microtiter wells, e) The substrate is added turning the coloration of the solution blue & f) The reaction is stopped by adding dilute sulfuric acid, turning the color of the solution yellow.

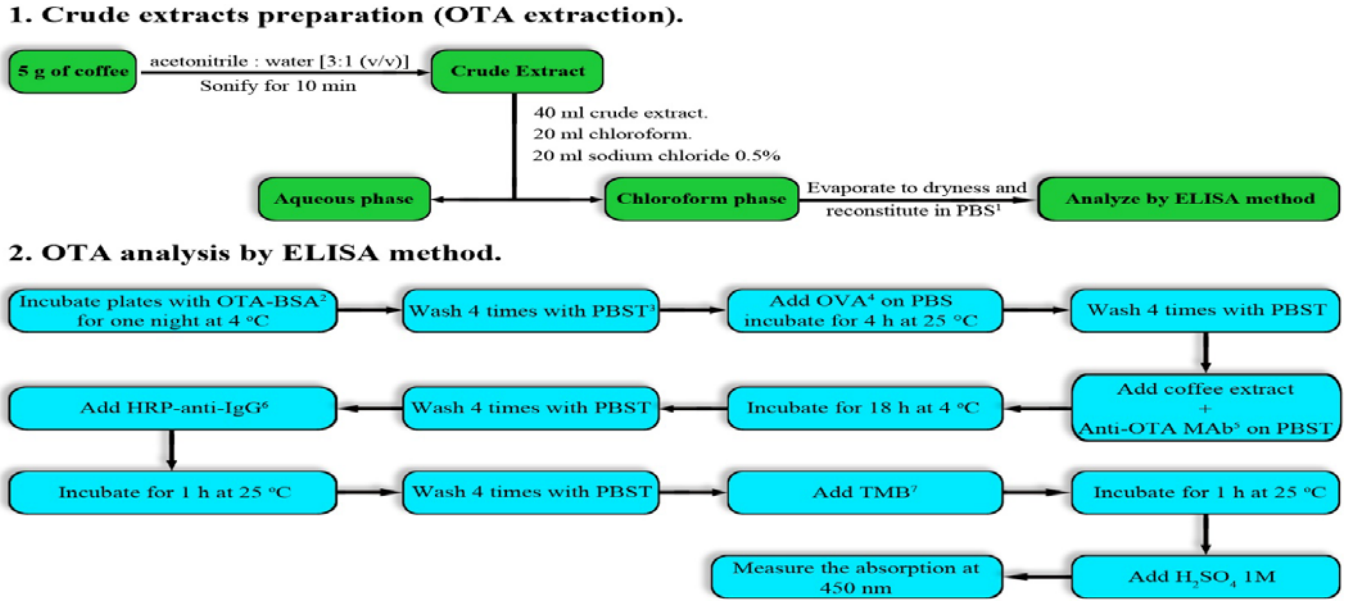


Fig. 3. OTA analysis in green, roasted, and instant coffee by the ELISA method.
[1] **PBS**: MeOH-phosphate buffered saline,
[2] **OTA-BSA**: OTA-enzyme conjugate, **BSA**: bovine serum albumin,
[3] **PBST**: PBS-0.05% Tween 20, [4] **OVA**: ovalbumin,
[5] anti-OTA MAb: anti-OTA antibody,
[6] **HRP-anti-IgG:** goat antibody against mouse immunoglobulin G,
[7] **TMB**: peroxide/tetramethylbenzidine.

7.3 DURING STORAGE

The raw coffee bean can be stored for up to three years under good storage conditions, that is, by reducing the bean humidity between 11-12%, which in turn prevents the proliferation of OTA-producing fungi [11,13,36].

However, according to Reference [30] "FAO, WHO and the United Nations Environment Program recommend reducing the humidity below 10%".

In addition, it is recommended that storage be carried out in polystyrene bags since they are less permeable than jute bags, which allow the reabsorption of moisture from the environment more easily [13]. For example, studies conducted by Reference [39] showed a difference of 5% moisture between coffee beans batches stored in polystyrene bags and those stored in jute bags. After 140 days of storage at 3 °C and a relative humidity of 59%, the coffee stored in polystyrene bags presented a humidity of 13.73 ± 1.09% while

the coffee stored in jute bags presented a humidity of 19.05 ± 1.25% [39].

However, it is also possible to prevent the production and even degrade the pre-existing OTA in stored grains through its exposure to gamma radiation. Experiments conducted by Reference [30] showed a negative correlation between the coffee beans' moisture content and the OTA concentrations when they are exposed to 10 kGy of gamma radiation. The obtained results show that after exposing the grains to gamma radiation (10 kGy) reductions in OTA levels of 5, 9, 20 and 90% were recorded when the grain moisture increased to 9, 10, 12 and 23% respectively [30]. The metabolite presence was practically eliminated when the humidity reached 58% [30].

The decreases registered in the OTA concentration through the exposure to gamma radiation are attributed to the grain sterilization in addition to the metabolite inactivation due to an increase in the free radicals' production [30].

In conclusion, the exposure of coffee beans to gamma radiation is an effective postharvest practice to degrade the OTA produced during the bean drying process (due to its high degree of humidity and contact with contaminated surfaces) in addition to sterilizing them, thus preventing its reinfection facilitated by the grain's hygroscopic nature during long storage periods [30].

7.4 DURING ROASTING

Despite its thermo-stability, OTA is normally degraded during coffee roasting (Table 4) [18,19,25] recording up to 90% of losses due to its thermal degradation, which is why coffee is not considered an important OTA dietary source [6,13].

However, the reduction of OTA concentrations during the grain roasting is conditioned by the concentrations present in the raw grain, the desired roasting degree, and the equipment used for roasting (toaster) [4,19]. For example, moderately roasted and well-roasted coffees (black coffees) show the lowest OTA concentrations [4]. However, due to the lack of standardization in the times and temperatures used during roasting, its presence in coffee can still be detected; The evidence suggests that coffees roasted for 20 min at 200 °C can show up to 12% OTA on a dry matter basis [30].

However, the use of high temperatures as a means of controlling OTA levels is an alternative that must be used with caution, since these can lead to the production of unpleasant compounds that can affect the organoleptic characteristics (e.g., colour, flavour, & aroma) of the final product [13] in addition to significantly reducing the antioxidant capacity of phenolic compounds which are degraded during prolonged grain roasting.

7.5 OTHER INDUSTRIAL PROCESSES

Grain decaffeination contributes to a significant OTA levels reduction [13,40] due to the action of polar organic solvents (e.g., dichloromethane and EtOAc) which tend to solubilize it, thus facilitating its removal (Table 3) [18].

Table 3. OTA concentration before and after decaffeination.

OTA Concentration	Samples (ppb)			Mean
Non-decaffeinated beans.	6.2	4.7	8.7	6.5
Decaffeinated bean.	1.3	1.7	0.9	1.2

Adapted from Reference [18]

Likewise, the polishing of raw coffee beans to remove the testa or spermoderm also contributes to the OTA levels reduction [18].

VIII. CONCLUSION

After the grain washing, its humidity oscillates between 52.7-53.5% [41] so it must be dried in order to be stored. With the endocarp (parchment) still attached, the grain is dried in the sun on terraces or in dryers (T ≤ 40 °C) to reduce its humidity by 12-14% [41-45] other authors suggest up to 10% [46] to avoid the development of fungal infections during storage, which are responsible for the accumulation of secondary metabolites (Aflatoxins & OTA) that impart negative sensory notes to the raw, roasted beans and coffee beverage.

For these reasons, the coffee bean must be processed as soon as possible and its storage should not exceed three years, even under good temperature and humidity conditions [47]. The storage and transport of the dry and deparchmented bean is carried out in the traditional jute bags made from vegetable fiber. Unfortunately, although such bags allow the free gas exchange between the grains and the surrounding environment under poor storage conditions (high relative humidity) they also allow the grains to absorb moisture which creates favourable conditions for the development fungal infections.

OTA's relative thermal degradation is the primary reason for coffee not to be considered an important dietary source; However, exposure to it, especially through the infusion, for protracted periods of time can cause the appearance of pathologies that affect both the liver and the kidneys. Unfortunately, in most coffee-producing countries there are no phytosanitary measures to monitor OTA concentrations in the coffee marketed within their borders, which is why it is preferable to consume processed coffees imported especially from the United States and Europe.

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Table 4. OTA content (μ kg^{-1}) in coffee samples inoculated with *Aspergillus ochraceus* before and after various degrees of roasting.

Roasting temperature	Light		Medium		Toasted	
	200 °C	**250 °C**	**200 °C**	**250 °C**	**200 °C**	**250 °C**
Coffee 1 (53.2 ± 0.3)						
Residue.	21.9 ± 0.3	25.2 ± 0.1	11.6 ± 1.1	12 ± 0.1	9 ± 0.1	10.6 ± 0.1
Percentage reduction.	58.7	52.6	78.3	77.4	82.9	80.1
Coffee 2 (16.6 ± 0.2)						
Residue.	n.e.	13.7 ± 0.2	n.e.	5.9 ± 0.1	n.e.	2.3 ± 0.3
Percentage reduction.		17.4		64.5		85.7

n.e., not evaluated. Adapted from Reference [20].

REFERENCES

[1] M. Lobeau, S. De Saeger, L. Sibanda, I. Barna-Vetró, and C. Van Peteghem, "Development of a new clean-up tandem assay column for the detection of ochratoxin A in roasted coffee," *Anal Chim Acta,* vol. 538, no. 1-2, pp. 57–61, May 2005.

[2] D. Vanesa, and P. Ana, "Occurrence of Ochratoxin A in coffee beans, ground roasted coffee and soluble coffee and method validation," *Food Control,* vol. 30, no. 2, pp. 675-78, April 2013.

[3] M. B. M. Ferraz, A. Farah, B. T. Iamanaka, D. Perrone, M. V. Copetti, et al., "Kinetics of ochratoxin A destruction during coffee roasting," *Food Control,* vol. 21, no. 6, pp. 872-77, June 2010.

[4] O. Castellanos-Onorio, O. Gonzalez-Rios, B. Guyot, T. A. Fontana, J. P. Guiraud, et al., "Effect of two different roasting techniques on the Ochratoxin A (OTA) reduction in coffee beans (*Coffea arabica*)," *Food Control,* vol. 22, no. 8, pp. 1184-88, August 2011.

[5] N. T. Program. *Ochratoxin A.*: U.S. Department of Health and Human Services; 2016 November 3. Available: http://tinyurl.com/4f9kxhtz.

[6] L. R. Batista, S. M. Chalfoun, C. F. Silva, M. Cirillo, E. A. Varga, and R. F. Schwan, "Ochratoxin A in coffee beans (*Coffea arabica* L.) processed by dry and wet methods," *Food Control,* vol. 20, no. 9, pp. 784-90, September 2009.

[7] M. H. Taniwaki, A. A. Teixeira, A. R. R. Teixeira, M. V. Copetti, and B. T. Iamanaka, "Ochratoxigenic fungi and Ochratoxin A in defective coffee beans," *Food Res Int,* vol. 61, no. pp. 161-66, July 2014.

[8] C. Chen, and F. Wu, "The need to revisit ochratoxin A risk in light of diabetes, obesity, and chronic kidney disease prevalence," *Food Chem Toxicol,* vol. 103, no. pp. 79-85, May 2017.

[9] A. Farah, "Coffee constituents," in *Coffee*, vol. Y.-F. Chu, Eds. Oxford, UK: Wiley-Blackwell, 2012. pp. 21-58.

[10] J. H. Hope, and B. E. Hope, "A review of the diagnosis and treatment of Ochratoxin A inhalational exposure associated with human illness and kidney disease including focal segmental glomerulosclerosis," *J Environ Public Health,* vol. 2012, no. pp. 835059, 2012.

[11] H. Ngabirano, "Enhancement of coffee quality through the prevention of mould growth: Start up experiences in Uganda," In: *COLLOQUE Scientifique International sur le Café, 18*, Helsinki, Finlandia, 1999. Available: https://goo.gl/td8qDn

[12] M. Suarez-Quiroz, O. Gonzalez-Rios, M. Barel, B. Guyot, S. Schorr-Galindo, and J.-P. Guiraud, "Study of ochratoxin A-producing strains in coffee processing," *Int J Food Sci Tech,* vol. 39, no. 5, pp. 501-07, May 2004.

[13] R. R. M. Paterson, N. Lima, and M. H. Taniwaki, "Coffee, mycotoxins and climate change," *Food Res Int,* vol. 61, no. pp. 1-15, July 2014.

[14] S. Amézqueta, S. Schorr-Galindo, M. Murillo-Arbizu, E. González-Peñas, A. López de Cerain, and J. P. Guiraud, "OTA-producing fungi in foodstuffs: a review," *Food Control,* vol. 26, no. 2, pp. 259-68, August 2012.

[15] A. Vecchio, V. Mineo, and D. Planeta, "Ochratoxin A in instant coffee in Italy," *Food Control,* vol. 28, no. 2, pp. 220-23, December 2012.

[16] P. Noonim, W. Mahakarnchanakul, K. F. Nielsen, J. C. Frisvad, and R. A. Samson, "Isolation, identification and toxigenic potential of Ochratoxin A-producing *Aspergillus* species from coffee beans grown in two regions of Thailand," *Int J Food Microbiol,* vol. 128, no. 2, pp. 197-202, December 10 2008.

[17] R. D. d. C. C. Bandeira, T. M. Uekane, C. P. d. Cunha, V. S. d. Cunha, J. M. Rodrigues, et al., "Development and validation of a method for detection and quantification of ochratoxin A in green coffee using liquid chromatography coupled to mass spectrometry," *Food Science and Technology,* vol. 32, no. 4, pp. 775-82, December 2012.

[18] W. Heilmann, A. G. Rehfeldt, and F. Rotzoll, "Behaviour and reduction of ochratoxin A in green coffee beans in response to various processing methods," *Eur Food Res Technol,* vol. 209, no. 3-4, pp. 297-300, August 1999.

[19] F. Malir, V. Ostry, A. Pfohl-Leszkowicz, J. Toman, I. Bazin, and T. Roubal, "Transfer of Ochratoxin A into

tea and coffee beverages," *Toxins (Basel),* vol. 6, no. 12, pp. 3438-53, December 17 2014.

[20] M. Suarez-Quiroz, B. D. Louise, O. Gonzalez-Rios, M. Barel, B. Guyot, et al., "The impact of roasting on the ochratoxin A content of coffee," *Int J Food Sci Tech,* vol. 40, no. 6, pp. 605-11, June 2005.

[21] B. Cramer, B. Osteresch, K. A. Munoz, H. Hillmann, W. Sibrowski, and H. U. Humpf, "Biomonitoring using dried blood spots: detection of ochratoxin A and its degradation product 2'R-ochratoxin A in blood from coffee drinkers," *Mol Nutr Food Res,* vol. 59, no. 9, pp. 1837-43, September 2015.

[22] S. Casal, T. Vieira, R. Cruz, and S. C. Cunha, "Ochratoxin A in commercial soluble coffee and coffee substitutes," *Food Res Int,* vol. 61, no. pp. 56-60, July 2014.

[23] A. Pfohl-Leszkowicz, and R. A. Manderville, "Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans," *Mol Nutr Food Res,* vol. 51, no. 1, pp. 61-99, January 2007.

[24] M. Tozlovanu, and A. Pfohl-Leszkowicz, "Ochratoxin A in roasted coffee from French supermarkets and transfer in coffee beverages: comparison of analysis methods," *Toxins (Basel),* vol. 2, no. 8, pp. 1928-42, August 2010.

[25] L. R. Batista, S. M. Chalfoun, G. Prado, R. F. Schwan, and A. E. Wheals, "Toxigenic fungi associated with processed (green) coffee beans (*Coffea arabica* L.)," *Int J Food Microbiol,* vol. 85, no. 3, pp. 293-300, August 25 2003.

[26] V. S. Ribeiro, A. E. Leitão, J. C. Ramalho, and F. C. Lidon, "Chemical characterization and antioxidant properties of a new coffee blend with cocoa, coffee silverskin and green coffee minimally processed," *Food Res Int,* vol. 61, no. pp. 39-47, 2014.

[27] G. Oliveira, D. M. da Silva, R. G. F. Alvarenga Pereira, L. C. Paiva, G. Prado, and L. R. Batista, "Effect of different roasting levels and particle sizes on Ochratoxin A concentration in coffee beans," *Food Control,* vol. 34, no. 2, pp. 651-56, December 2013.

[28] N. Martinez-Saez, A. T. Garcia, I. D. Perez, M. Rebollo-Hernanz, M. Mesias, et al., "Use of spent coffee grounds as food ingredient in bakery products," *Food Chem,* vol. 216, no. pp. 114-22, Feb 1 2017.

[29] S. L. Leong, L. T. Hien, T. V. An, N. T. Trang, A. D. Hocking, and E. S. Scott, "Ochratoxin A-producing Aspergilli in Vietnamese green coffee beans," *Lett Appl Microbiol,* vol. 45, no. 3, pp. 301-06, September 2007.

[30] S. Kumar, A. Kunwar, S. Gautam, and A. Sharma, "Inactivation of *A. ochraceus* spores and detoxification of ochratoxin A in coffee beans by gamma irradiation," *J Food Sci,* vol. 77, no. 2, pp. T44–T51, February 2012.

[31] E. de Fatima Rezende, J. G. Borges, M. A. Cirillo, G. Prado, L. C. Paiva, and L. R. Batista, "Ochratoxigenic fungi associated with green coffee beans (*Coffea arabica* L.) in conventional and organic cultivation in Brazil," *Braz J Microbiol,* vol. 44, no. 2, pp. 377-84, 2013.

[32] R. Krska, P. Schubert-Ullrich, A. Molinelli, M. Sulyok, S. MacDonald, and C. Crews, "Mycotoxin analysis: an update," *Food Addit Contam Part A Chem Anal Control Expo Risk Assess,* vol. 25, no. 2, pp. 152-63, February 2008.

[33] S. Fujii, E. Y. S. Ono, R. M. R. Ribeiro, F. G. A. Assunção, C. R. Takabayashi, et al., "A comparison between enzyme immunoassay and HPLC for ochratoxin A detection in green, roasted and instant coffee," *Braz Arch Biol Technol,* vol. 50, no. 2, pp. 349-59, March 2007.

[34] R. Aparicio, and R. Aparicio-Ruiz, "Authentication of vegetable oils by chromatographic techniques," *J Chromatogr A,* vol. 881, no. 1-2, pp. 93-104, June 9 2000.

[35] M. Z. Zheng, J. L. Richard, and J. Binder, "A review of rapid methods for the analysis of mycotoxins," *Mycopathologia,* vol. 161, no. 5, pp. 261-73, May 2006.

[36] C. Alimentarius, "Código de prácticas para prevenir y reducir la contaminación de Ocratoxina en el café," *CAC/RCP,* vol. 69, no. pp. 19, 2009.

[37] C. Lambot, J. C. Herrera, B. Bertrand, S. Sadeghian, P. Benavides, and A. Gaitán, "Cultivating coffee quality - Terroir and agro-ecosystem," in *The craft and science of coffee*, vol. B. Folmer, Eds.: Academic Press, 2017. pp. 17-49.

[38] B. T. Iamanaka, A. A. Teixeira, A. R. R. Teixeira, M. V. Copetti, N. Bragagnolo, and M. H. Taniwaki, "The mycobiota of coffee beans and its influence on the coffee beverage," *Food Res Int,* vol. 62, no. pp. 353-58, August 2014.

[39] C. F. Silva, L. R. Batista, L. M. Abreu, E. S. Dias, and R. F. Schwan, "Succession of bacterial and fungal communities during natural coffee (*Coffea arabica*) fermentation," *Food Microbiol,* vol. 25, no. 8, pp. 951-57, December 2008.

[40] A. Pietsch, "Decaffeination - Process and quality," in *The craft and science of coffee*, vol. B. Folmer, Eds.: Academic Press, 2017. pp. 225-43.

[41] J. R. Sanz-Uribe, Yusianto, S. N. Menon, A. Peñuela, C. Oliveros, et al., "Postharvest processing - Revealing the green bean," in *The craft and science of coffee*, vol. B. Folmer, Eds.: Academic Press, 2017. pp. 51-79.

[42] A. Tarzia, M. B. Dos Santos Scholz, and C. L. De Oliveira Petkowicz, "Influence of the postharvest

processing method on polysaccharides and coffee beverages," *Int J Food Sci Technol,* vol. 45, no. 10, pp. 2167-75, October 2010.
[43] A. J. Turnbull, "Coffee," *Nutr Bull,* vol. 6, no. 3, pp. 153-65, September 1981.
[44] A. Farah, and T. Ferreira dos Santos, "The coffee plant and beans," in *Coffee in health and disease prevention*, vol. San Diego: Academic Press, 2015. pp. 5-10.
[45] T. R. Lingle, and S. N. Menon, "Cupping and grading - Discovering character and quality," in *The craft and science of coffee*, vol. B. Folmer, Eds.: Academic Press, 2017. pp. 181-203.
[46] P. S. Murthy, and M. Madhava Naidu, "Sustainable management of coffee industry by-products and value addition - a review," *Resour Conserv Recycl,* vol. 66, no. pp. 45-58, September 2012.
[47] C. Yeretzian, I. Blank, and Y. Wyser, "Protecting the flavors - Freshness as a key to quality," in *The craft and science of coffee*, vol. B. Folmer, Eds.: Academic Press, 2017. pp. 329-53.