beverages

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Review

Occurrence of Ochratoxin A in Coffee: Threads and Solutions—A Mini-Review

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Abstract: Ochratoxin A (OTA) is a widespread bioactive extrolite from secondary metabolism of fungi which presence in foods like coffee is of public health concern, particularly for heavy drinkers. Coffee is one of the most consumed and appreciated non-alcoholic beverage in the world. Its production from the plantation to the coffee cup involves several steps that would determine the final concentration of OTA in the beverage. This review gives an overview of OTA contamination in roasted coffee beans in different countries and mitigation strategies for OTA reduction.

Keywords: ochratoxin A; coffee; mycotoxin; threads; treatment

1. Coffee as a Unique Beverage

Coffee is one of the most popular beverages nowadays all over the world, with an annual global production of 10.4×10^6 tons and it plays an important cultural and economic role [1]. It contains more than 1500 chemical components, being responsible for sensorial and functional properties of the coffee beverage, the second most valuable legally traded commodity [2–4]. A recent study by Samoggia et al. (2018) indicated that consumers' preferences for a specific coffee type mainly depended on sensory aspects like taste and aroma and functional motives to improve alertness and physical performance, beside habits and traditions of the consumer [5]. Coffee can be consumed as a hot or cold beverage, usually as the result of an infusion of properly processed coffee beans, being consumed per se as part or as the last component of meals or as an ingredient in snacks. It is, generally, produced from two biologically dissimilar species the *Coffea arabica* and *Coffea canephora* var. *robusta*, representing 60% and 40%, respectively, of world production [6]. Coffee from *Coffea arabica* (Arabica) and *Coffea canephora* var. *robusta* (Robusta) have several differences not only in terms of botanical, genetic, and morphological features of bean but also in their chemical composition and environmental conditions [7]. For example, while Arabica is adapted to lower temperatures and reduced humidity characteristics of high altitudes, Robusta is cultivated from sea level up to 1000 m; caffeine concentration endogenously present in the green coffee extracts of Robusta, often, is double that of Arabica, being dependent also of geographical origin and producing a drink with pronounced bitterness [6,7]. The tocopherols contents of Arabica are higher than Robusta and while 90% of tocopherols remain after roasting in the case of Arabica, a degradation of approximately 25% is observed for β-tocopherol in Robusta [8]. Recently, it was reported that the roasting procedure affected differentially coffee varieties in what concerns to antimutagenic activity, while no effect was observed in Robusta varieties, roasting enhances time-dependently the antimutagenicity of the Brazil variety (Arabica) [9].

Coffee is a unique beverage with healthy properties that make it a functional food due to its content in chlorogenic acids, caffeine, trigonelline, diterpenes and tocopherols present in raw

beans. Therefore, the coffee extracts exhibited free radical scavenging and induced detoxification enzymes [9,10]. Additionally, beneficial effects of coffee such as anti-mutagen, inductor of the activation of DNA repair, as well as in the decrease of mortality from neurological and cardiovascular diseases, and diabetes type II has been reported [10,11]. Despite the literature support the role of coffee as having an immunomodulatory action, it seems to be disease-specific [11].

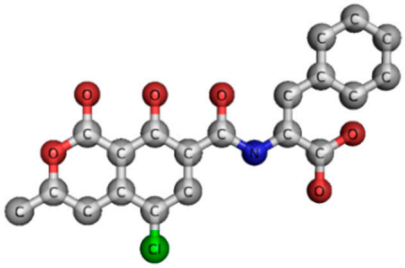
Recently, a study investigated the hypothesis that habitual coffee consumption is associated with lower risk of frailty, physical function impairment, and disability in women with aged 60 years or older [12]. The conclusion was that habitual coffee consumption of two or more cup of coffee/day, in older people, was not associated with increased risk of functional impairment, and it can be positive in the case of individuals with diabetes, hypertension or obesity [12].

2. Ochratoxin A as a Coffee Contaminant

2.1. Ochratoxin A

The mycotoxin ochratoxin A (OTA) is a low molecular weight ubiquitous secondary metabolite, a weak organic acid consisting of an amino acid phenylalanine and a dihydro-isocoumarin linked by a peptide bond (Table 1).

Table 1. Structure, formula and physical characteristics of ochratoxin A (OTA).

Name	***N*-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)carbonyl]-L-phenylalanine**	Reference
Chemical structure		
Formula	$C_{20}H_{18}ClNO_6$	[13]
Molecular weight	403.8 g·mol^{-1}	[13]
Melting point	168–173 °C (drying for 1 hour at 60 °C)	[14]
	159 °C (recrystallized from benzene-hexane)	[15]
	169 °C (recrystallized from xylene)	[16]
$[\alpha]^{21}_D$	−46.8° (c = 2.65 mmol/L in chloroform)	[14]
$[\alpha]^{20}_D$	−118° (c = 1.1 mmol/L in chloroform)	[16]
λ_{max}	214 nm ($\varepsilon = 37.2 \times 10^{-3}$ L·mol^{-1}·cm^{-1}) 282 nm ($\varepsilon = 0.89 \times 10^{-3}$ L·mol^{-1}·cm^{-1}) 332 nm ($\varepsilon = 6.33 \times 10^{-3}$ L·mo^{-1}·cm^{-1}) (c = 0.0281 mmol/L in methanol)	[14]
pKa	4.2–4.4 and 7.0–7.3	[17]

OTA is a white crystalline, odorless, solid compound with poor aqueous solubility, but moderately soluble in chloroform, methanol, ethanol and xylene. It is heat-stable, being described as the most common member in group of ochratoxins [18,19]. The group of ochratoxins is principally formed by OTA, ochratoxin B (OTB) and ochratoxin C (OTC). From a chemical perspective, while OTA is chlorinated, ochratoxin B is not chlorinated, and ochratoxin C is the ethyl ester of OTA [18]. Although OTA appear to be overall the most toxic, from recent investigations a different and more detailed picture have resulted with cell models showing in lower or similar toxicity of OTB compared to OTA; while there seems no doubt that OTB is less toxic in vivo compared to OTA, similar acutely cytotoxic in vitro is observed. Meanwhile OTC seems to be equally acutely toxic in vivo and in vitro compared to OTA [20]. Nowadays, based on the metabolism of animals and humans, the number of molecules

belonging to this group has increased substantially, especially on OTA derivatives. Examples of this are hydroxylated, conjugated or metabolites where phenylalanine moiety is absent [21]. Despite its stability, OTA can be transformed mainly to metabolites, which were considered to have low or no toxicity (Figure 1) [22–25]. Today's knowledge on comparative toxicity of OTA and its metabolites shows that ochratoxin α (OTα) is approximately 100 fold less toxic than OTA; 10-hydroxyochratoxin A (10-OH OTA) is non-genotoxic, and conjugated ochratoxin forms are, as so far, not toxic; meanwhile the 4-hydroxyochratoxin A (4-OH OTA) shows similar toxicity, and lactone-opened OTA (OP-OTA) could be even more toxic than OTA [20].

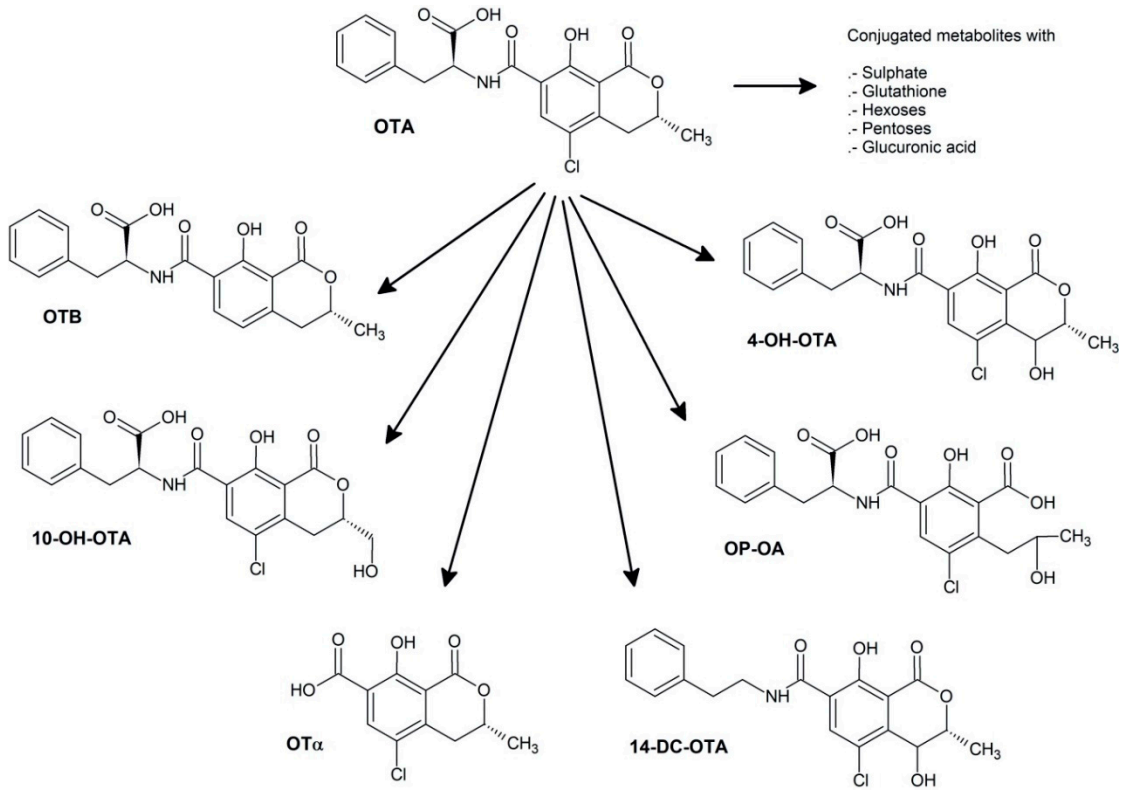


Figure 1. Structures of ochratoxin A (**OTA**) and transformation bioproducts ochratoxin B (**OTB**), 10-hydroxyochratoxin A (**10-OH-OTA**), ochratoxin α (**OTα**), 14-decarboxy-ochratoxin A (**14-DC-OTA**), lactone-opened OTA (**OP-OA**), 4-hyroxyochratoxin A (**4-OH-OTA**) and OTA conjugated metabolites adapted from [23–25].

This mycotoxin was reported for the first time in 1965 [16] and nine years later it was described in coffee [26]. Although humans can be exposed to OTA by inhalation or dermal contact, dietary intake is the principal source of OTA for humans because it is found in a variety of foods, like for example; maize, wheat, rice, sorghum, barley, oats, rye, bread, flour, pasta, infant cereals, grapes, apples, peaches, pears, strawberries, oranges, figs, mangoes, tomatoes, wine, watermelons, nuts, sesame seeds, rapeseed, spice, coffee beans, cocoa, peanuts, chickpeas, soybeans, milk and milk-based baby formulae, cheese, eggs, yam, garlic, onions, potatoes, pork, fish, poultry, dried beans, jerky, smoked or salted dried fish, and raisins. More recently, its presence was also detected in bottled water [27], food coloring agents and plant food supplements [28]. Therefore, avoiding the consumption of OTA is nearly impossible, having as a consequence the presence of low but detectable levels of OTA in human blood, coffee being one important source. In the 1970s it was reported the first studies that identified the presence of OTA in human blood in the Balkans [29]. In fact, OTA can be found in human body due to its long half-life of 35 days [30]. In the review of Malir et al. (2016) it is compiled published data on OTA in

human blood samples from healthy persons, and concentrations higher than 1.0 µg/L were observed in several countries.

OTA displays a vast toxicity, including neurotoxic, teratogenic, immunotoxic, carcinogenic, hepatotoxic, embryotoxic and especially nephrotoxic in laboratory and farm animals [19,21]. Toxicokinetics data showed that the susceptibility to cancer depending on the species and sex of the tested animals [19,31]. The available kinetic data of OTA for humans is rather limited than for animals, but gender (male population showed higher values), and particularly season (in summer the highest values were obtained) and geographic location (identify with dietary habits of each particular region) appear to be the most relevant factors that influencing OTA concentrations in plasma [32].

In 1993, the International Agency for Research on Cancer has evaluated OTA as a possible human carcinogen (Group 2B) based on inadequate evidence in humans for the carcinogenicity of OTA [33]. Surprisingly, chronic exposure to low OTA doses could be even more severe effects than acute exposure to a high dose [21,34]. It is generally accepted that the kidney is the classical target organ of OTA [34]. The toxin at nanomolar concentrations in renal cells may be regarded as a modulator of cellular signaling, since it interacts with certain cellular key targets resulting in specific alterations of cell function and/or phenotype and even may stable re-program the cells. At these concentrations OTA can induce apoptosis in culture cells, while necrosis has only a minor role in cellular death [34]. Later, Schwerdt showed that exposure to very low OTA concentrations (0.3–10 nm) for prolonged time (up to 14 days) led not only to cell hypertrophy, but also affect the cellular fate in human proximal tubule cells [35]. Moreover, findings of increased secretion of fibronectin, NF-κB activity and modified collagen pattern, was proposed for OTA induced tubule-interstitial fibrosis which is frequently observed in Balkan endemic nephropathy [35]. Toxicogenomic studies of Balkan nephropathy on the human genes that influence susceptibility to OTA, lead to the hypotheses about the association between Balkan nephropathy to be produced by OTA [36]. Nowadays, it is assumed that OTA is the probable causal agent in the development of Balkan endemic nephropathy, a chronic tubulointerstitial kidney disease that may lead to kidney tumors and irreversible kidney failure [13,37].

An in vitro study conducted in human fetal gastric epithelium cells (GES-1) showed that OTA could induce a G2 arrest, increasing intracellular reactive oxygen species (ROS) production and consequently inducing DNA damage. The oxidative DNA damage elevated the levels of 8-hydroxydeoxyguanosine and DNA double-strand breaks. It was also observed that OTA exposure induced the phosphorylation of the ataxia telangiectasia-mutated (ATM) protein suggesting that OTA-induced oxidative DNA damage triggered the ATM-dependent pathways [38]. Interestingly, among normal human cells, OTA could induce a G1 arrest in human peripheral blood mononuclear cells [39].

At present, although there is insufficient data regarding in utero exposure and the fetotoxicity of OTA, it is known that the level of OTA in human umbilical cord blood is higher than maternal blood at the time of delivery. Considering that human blood samples from healthy individuals could be relatively high as described above, it is recommended that OTA exposure of pregnant women should kept as low as possible in order to reduce the possibility to develop cancer early in life, or even increase susceptibility to chronic disease or cancer later in life [40].

A recent data analysis on OTA suggested a strong evidence of its carcinogenicity in humans [21], probably due to induction of oxidative stress by OTA [13] or through oxidative DNA lesions coupled with direct DNA adducts via quinone formation [17]. This last theory concurs with the Maaroufi and Arlt studies, which detected OTA-DNA adducts in tissues of humans exposed to OTA [37,41]. On the other hand, other studies postulated that OTA induced DNA damage in association with ROS production in several cell types including human kidney (HK-2), proximal tubular cells and colonic adenocarcinoma cell line (CaCo-2), as well as peripheral blood mononuclear cells and hepatoma cell lines (HepG2) [38,39,42–44]. Indeed, it is believed that OTA genotoxicity is through oxidative metabolism, playing and important role in OTA-mediated carcinogenesis [17]. Furthermore, a model for the role of miR-122, the most abundant liver micro-RNA (miRNA), in OTA-induced hepatocyte apoptosis in vivo and in vitro was proposed [45].The expression of miR-122 in HepG2 was upregulated when treated

with OTA. miR-122 is a primary effector of OTA-induced hepatocyte apoptosis. The mechanism across CCNG1/P53 and Bcl-w/caspase-3 pathways was described [45].

The mechanism of toxicity of OTA is complex and new data are necessary to better understand all the events implicated. Nevertheless, taking into account the previous and recently available data, there are voices in the scientific community that propose upgrade its carcinogenicity from Group 2B to at least Group 2A (probably carcinogenic to humans) [20,35,46–48] or even Group 1 (carcinogenic to humans) [21].

2.2. Fungal Contamination and Coffee

Green coffee beans are prone to fungal attack. Among these microorganisms, *Aspergillus* species such as *Aspergillus carbonarius*, *A. niger*, *A. ochraceus* and *A. westerdijkiae* are the most widely OTA producers in tropical and semi-tropical coffee plantations [7,49]; meanwhile in temperate regions *Penicillium* species such as *P. verruculosum*, *P. brevicompactum*, *P. crustosum*, *P. olsonii* and *P. oxalicum* are usually found [50,51]. Recently, a strain of *Byssochlamys spectabilis* isolated from roasted coffee from Mexico markets was able to produce OTA [52]. An EU study confirmed that green coffee is a raw material susceptible to OTA contamination [53]. The mycotoxigenic potencial to contaminate the coffee beans depends on several factors such as species and strain of fungus, water activity, environmental (climatic conditions, storage and transportation) and processing (wet and dry processing) conditions [7,54–56]. In fact, OTA has been reported at different stages of the food chain, indicating that fungus colonization may occur before or after coffee harvest, depending on environmental or storage conditions, particularly in the case that coffee seeds are not dried to a safe moisture percentage or if rehydrated during any of the phases of drying, storage or transportation [55]. Factors such as the adequate cherry maturation using normal water, fertilizers and maintenance, as well as harvest only ripe cherries which were not in contact with soil to prevent microorganism contamination and the appearance of black and sour defective beans, and the reduction of defects at purchasing are important points in green coffee production chain. Finally, the transport should be done to well-dried green coffee (10–12% MC) and storage conditions should assure an atmosphere at 50–70% RH and a temperature below 20 °C to keep OTA levels generally low [55,57]. Therefore, the critical steps for OTA accumulation are considered to be the harvest and the postharvest handling of coffee cherries [7]. Additionally, OTA is stable under acidic conditions, high temperatures and pressure steam sterilization, making difficult the control of its sanitary quality in order to minimize the risk of its intake from coffee [19,58]. Taking into account the risk that OTA presents to human health, the European Union has developed regulations and fixed a maximum level for OTA in the roasted coffee bean and ground roasted coffee of 5 µg/kg of ochratoxin A [59]. Additionally, based on the toxicological data, the European Safety Authority fixed a tolerable weekly intake (TWI) of 120 ng/kg body weight [60].

3. Can Ochratoxin A Reduce Coffee Consumption?

An EU study reported that there is a number of data sufficiently high to provide sound information on the green and processed coffee contamination by OTA [53]. The percentage of positive samples was 36% and 46% for green coffee beans and roasted coffee, respectively, among EU member states, with average of OTA contamination levels of 3.641 µg/kg and 1.092 µg/kg, respectively. Nevertheless, countries like Germany, Greece, Italy, Spain and Netherlands reported positive samples for roasted coffee with at least 5.000 µg/kg of OTA [53]. The occurrence of OTA in roasted coffee bean has also been reported by other studies in concentrations ranging between 0.09 µg/kg and 12.1 µg/kg (Table 2). The highest level of OTA (12.1 µg/kg) was found in a sample from Germany [61]. Although in countries like Argentina, Brazil, France and Portugal, OTA was also detected in samples exceeding the maximum level established by EU, the number of samples was reduced comparing to sampling [62–66]. Despite of the lack of consensus in literature regarding the percentage of toxin that is transferred into beverage during brewing, it is known that home-processing methods can influence the OTA concentration in coffee brews [67–71]. It was reported that a reduction of approximately 50% of the toxic potential of

roast and ground coffee was obtained in expresso coffee, meanwhile in moka and auto-drip brews, a lower efficacy was achieved [69]. Malir noticed that the amount of OTA transfer into beverages was dependent on raw material contamination, time of contact, amount of ground coffee and water used as well as temperature of water and pH of the beverages. In the study conducted by this research group, values ranged from 22.3% to 66.1% of OTA transfer into coffee were found [67].

Table 2. Occurrence of OTA in roasted and/or ground roasted coffee by country.

Country	*n*	% Positive	Average (μg/kg)	Range of OTA (μg/kg)	Reference
Argentina	24	54	1.00	0.11–5.78	[64]
Brazil	34	68	0.9	0.3–6.5	[65]
Brazil	16	31		0.09–9	[62]
Canada	71	59	0.6	0.1–2.3	[72]
Chile	24	100	0.47	tr–0.84	[3]
France	30	100		tr–11.9	[66]
Germany	490	44	1.48	<0.15–12.1	[61]
Hungary	38	58	0.5	0.17–0.91	[73]
Italy	30	27	0.27	tr–<5.0	[74]
Portugal	11	27	1.66	0.71–10.31	[63]
Spain	72	49	1.05	1.21–4.21	[75]
UK	20	85	0.6	0.2–2.1	[76]

tr, traces (values of ochratoxin A between LOD and LOQ).

The consumption of this pleasant functional food due its taste and aroma could be taken with caution due to the presence of OTA, especially to high consumers where an intake of more than four coffees by day is a possible scenario. Moreover, if coffee beverage is prepared with approximately 7 g of ground coffee for 100 mL of water, the intake of a high consumer weighing 60 kg could corresponds to half of tolerate daily intake of ochratoxin A set up by JECFA, which made OTA intake via coffee beverage not negligible [66]. Nowadays, it is estimated that coffee accounts for approximately 12% of the total intake of OTA, being considered the third source of OTA exposure [3,77]. In a comparison study to assess human exposure to the OTA and 2'R-ochratoxin A (2'R-OTA, previously named 14R-Ochratoxin A), a product from thermal degradation, between coffee and noncoffee drinkers showed that no correlation exist between the amounts of coffee consumption and OTA or 2'R-OTA concentrations. However, 2'R-OTA was only detected in blood of coffee drinkers [78].

4. Strategies to Reduce Ochratoxin A

4.1. Coffee Roasted

Coffee roasting, one of the most important stages to obtain the brew, where non-enzymatic browning occurs depending on time and temperature that can be applied to green coffee samples, is responsible for the characteristics of the final product like the pleasant flavor as a consequence of chemical reactions. Besides, this interesting effect that make coffee an unique beverage, the roasting process is described as a step that decreases OTA levels [7,49,64,79,80]. Indeed, until 1988, it was believed that the roasting process completely degraded this mycotoxin. Tsubouchi et al. reported for the first time the occurrence of ochratoxin A in coffee brews [81]. Several studies have been published from then till now, particularly, using coffee "naturally contaminated" or "artificially contaminated" in order to demonstrate the effect of roasting on the OTA concentration (Table 3).

Table 3. Physical approaches for detoxification of OTA.

Method	% Reduction	Remarks	Reference
Roasting	67 63 74 53 84	Temp = 470 °C; Time = 2.5 min; batch size = 5 kg Temp = 490 °C; Time = 2.5 min; batch size = 5 kg Temp = 490 °C; Time = 4 min; batch size = 10 kg Temp = 400 °C; Time = 10 min; batch size = 15 kg Temp = 425 °C; Time = 10 min; batch size = 15 kg $[OTA]_{initial}$ = 4.9 µg/kg; (Gothot RN 100 pilot size roaster)	[82]
Roasting	77.9[a]; 64.1[b] 90.5[a]; 88.7[b] 96.8[a]; 95.1[b]	Temp = 450 °C; Time = 6 min; batch size = 3 kg Temp = 450 °C; Time = 7 min; batch size = 3 kg Temp = 450 °C; Time = 9 min; batch size = 3 kg [a]$[OTA]_{initial}$ = 29.30 µg/kg; [b]$[OTA]_{initial}$ = 9.64 µg/kg; (Laboratory roaster, model 500, STA)	[80]
Roasting	31.1	Temp = 180 °C; Time = 10 min; $[OTA]_{initial}$ = 29.36 µg/kg (Normal coffee roaster)	[83]
Roasting	79.1	Temp = 260 °C; Time = 5 min; batch size = 0.5 kg; $[OTA]_{initial}$ = 2.54 µg/kg; (Precision Coffee Roaster model, Hearthware Home Products)	[69]
Roasting	25.1[a]; 15.1[b] 59.5[a]; 67.9[b] 87.0[a]; 89.6[b] 97.2[a]; 95.1[b] 7.4[a]; 9.3[b] 36.0[a]; 30.9[b] 72.4[a]; 64.3[b] 77.6[a]; 73.4[b]	Temp = 230 °C; Time = 6 min; batch size = 0.2 kg Temp = 230 °C; Time = 9 min; batch size = 0.2 kg Temp = 230 °C; Time = 12 min; batch size = 0.2 kg Temp = 230 °C; Time = 15 min; batch size = 0.2 kg (Rotating cylinder- Probat-Werne type RE1 pilot roaster) Temp = 230 °C; Time = 0.9 min; batch size = 0.2 kg Temp = 230 °C; Time = 1.7 min; batch size = 0.2 kg Temp = 230 °C; Time = 2.6 min; batch size = 0.2 kg Temp = 230 °C; Time = 3.5 min; batch size = 0.2 kg (Fluidized bed - Neuhaus Neotec pilot roaster) [a]$[OTA]_{initial}$ = 5.3 µg/kg; [b]$[OTA]_{initial}$ = 57.2 µg/kg	[49]
Gamma radiation	5 9 20 90 ≈100	a_w = 0.59; Dried green coffee beans; a_w = 0.65; Incubated at RH 76 for 72 h; a_w = 0.71; Incubated at RH 99 for 72 h a_w = 0.82; Steamed for 1 h a_w = 0.93; Soaked for 20 h $[OTA]_{initial}$ = 50 ppb; radiation = 10 kGy (Gamma Chamber 5000)	[84]
High-pressure	0 54.6 93.2 96.2 14.6 43.6 93.2 96.0 19.1 45.7 91.9 95.5 26.9 36.3 90.8 94.4	P = 50 MPa; Time storage = 10 days; $[OTA]_{initial}$ = 4.4 µg/kg P = 200 MPa; Time storage = 10 days; $[OTA]_{initial}$ = 3.9 µg/kg P = 400 MPa; Time storage = 10 days; $[OTA]_{initial}$ = 4.4 µg/kg P = 600 MPa; Time storage = 10 days; $[OTA]_{initial}$ = 4.5 µg/kg P = 50 MPa; Time storage = 20 days; $[OTA]_{initial}$ = 4.4 µg/kg P = 200 MPa; Time storage = 20 days; $[OTA]_{initial}$ = 3.9 µg/kg P = 400 MPa; Time storage = 20 days; $[OTA]_{initial}$ = 4.4 µg/kg P = 600 MPa; Time storage = 20 days; $[OTA]_{initial}$ = 4.5 µg/kg P = 50 MPa; Time storage = 30 days; $[OTA]_{initial}$ = 4.4 µg/kg P = 200 MPa; Time storage = 30 days; $[OTA]_{initial}$ = 3.9 µg/kg P = 400 MPa; Time storage = 30 days; $[OTA]_{initial}$ = 4.4 µg/kg P = 600 MPa; Time storage = 30 days; $[OTA]_{initial}$ = 4.5 µg/kg P = 50 MPa; Time storage = 50 days; $[OTA]_{initial}$ = 4.4 µg/kg P = 200 MPa; Time storage = 50 days; $[OTA]_{initial}$ = 3.9 µg/kg P = 400 MPa; Time storage = 50 days; $[OTA]_{initial}$ = 4.4 µg/kg P = 600 MPa; Time storage = 50 days; $[OTA]_{initial}$ = 4.5 µg/kg (BaoTou KeFa High Pressure Technology Co.)	[85]

One of these interesting studies investigated the effect of roasting conditions on the reduction of OTA during roasting [82]. Roasting conditions varied from clearly roasting in commercial scale equipment to clearly outside the usually roasting practice, and no statistically significant differences between the color and the levels of the applied roasting were observed. In this study a mean value for OTA reduction of 68% on mycotoxin content was achieved. Nevertheless, the highest OTA reduction (84%) was found for the combination of long plus dark roasting, indicating that statistical significance could be obtained in darker colors and longer times of treatment [82]. The influence of roasting process on the OTA levels in coffee beans was also reported by Romani and collaborators. The reduction of OTA

content was a function of the severity of the thermal treatment and seems to be generally related to the initial OTA concentration (high percentages of OTA reduction in the highest contaminated samples) [80]. The effect of two different roasting techniques, rotating cylinder and fluidized bed, on OTA levels of green coffee beans (*Coffea arabica*) artificially contaminated with *Aspergillus westerdijkiae* have been reported [49]. A reduction on OTA levels above 96% and 75% was achieved under rotating cylinder and fluidized bed at the same roasting degree, respectively [49]. In a study conducted by Drunday and Pacin, the average of OTA levels in green coffee was almost six times greater than roasted ground coffee samples from markets located in Buenos Aires Province [64]. On contrary, Tsubouchi et al. described roasting at 200 °C during 10 and 20 min as an ineffective treatment in the reduction of OTA levels of contaminated coffee beans, since only a decrease of 0–12% was observed [71]. The discrepancy between these findings is difficult to explain but the OTA reduction during roasting may be due to: Epimerization at the C3 position [70,83], thermal degradation with possible involvement of moisture and a physical removal of OTA with chaff [79,83]. In a model system simulating degradation reactions of coffee roasting two products were identified, 14-(*R*)-ochratoxin A the major compound formed and 14-decarboxy-ochratoxin A at traces levels [24]. Detoxification of OTA by the formation of ochratoxin A saccharide esters and ochratoxin α amide during the coffee roasting was proposed [22,23]. When OTA is submitted to temperatures of 200 °C and above, a partial decomposition of the mycotoxin was observed and the ochratoxin α amide was formed as the thermal degradation product by a cleavage between the carbon atom C-14 and the nitrogen atom [22]; while in the case of ochratoxin A saccharide esters, a ester linkage between the carboxyl group of OTA and the primary hydroxyl group of the carbohydrates of the matrix was demonstrated [23]. Furthermore, ochratoxin α amide can be formed by photolytic degradation during the exposure of OTA to blue and white light, suggesting light treatment as a possible treatment to destroy OTA [22].

4.2. Coffee Composition: Caffeine

Coffee contains caffeine, 1,3,7-trimethylxanthine, a psychoactive molecule and one of the most important active compounds with antioxidant properties, being the major water-soluble component of coffee. Caffeine is described as a compound able to reduce type 2 diabetes mellitus disease and incidence of cancer, as well as protect against Parkinson´s disease and prevent bronchopulmonary dysplasia in premature neonates [86–89]. Meanwhile, caffeine concentration varies substantially, depending on coffee plant species, method of bean roasting, as well as drinks preparation [2]. Several studies have suggested that caffeine content could be a strategy to prevent OTA in green coffee bean, based on the fact that its presence reduces OTA production [90–92]. Buchanan et al. (1982) reported the effect of caffeine on growth and OTA production by *Aspergillus ochraceus* NRRL 3174 in YES medium. Caffeine was less inhibitory to growth than OTA synthesis. A dose related decrease in OTA accumulation was observed. A concentration of 3 mg/mL of caffeine inhibited OTA almost completely (98%). Despite different OTA inhibition percentages, a similar trend in AMY medium supplemented with 4 mg/mL of caffeine after 14 days of incubation was observed for mycotoxigenic strains, namely *A. flavus* PC65, *A. versicolor* XVII/19, *Penicillium urticase* NRRL 989, *P. roqueforti* M251 and *P. expansum* NRRL 973 [91]. In contrast, *Aspergillus ochraceus* strains S-235-100 isolated from green coffee beans produced higher amounts of OTA when grown on YES medium supplemented with 0.5% and 1.0% of caffeine, reaching a maximum after 15 to 20 days of incubation [92]. However, in the same study, it was investigated in solid medium the effect of caffeine on OTA production by 15 strains of *A. ochraceus*, three strains of *A. elegans* and one of *A. sclerotiorum*, and concluded that caffeine, generally, had little or no effect on OTA production by the majority of *A. ochraceus* strains, but did inhibit toxin production by *A. elegans* and *A. sclerotiorum* strains. While, Nehad et al. (2005) reported that caffeine at concentrations of 1.0% and 2.0% completely prevented OTA production by *A. ochraceus* in YES medium [83]. Recently, in a study conducted by Akbar et al. (2016) demonstrated that OTA production by strains from *Aspergillus* section *Circumdati* and *Aspergillus* section *Nigri* groups isolated from coffee had differential tolerance to concentrations of caffeine. If low concentrations, less than 0.5%, of caffeine were inhibitory to OTA

production by some strains, namely *A. carbonarius*, *A. westerdijkiae* and *A. niger*, at least 1.5% of caffeine was needed for *A. steynii* inhibition; while 4% of caffeine was required for *A. ochraceus*. However, OTA production was stimulated with 3% of caffeine [90]. In summary, it appears that caffeine has different effects on OTA production depending on its concentration but, particularly, of mycotoxigenic species that have the ability to contaminate coffee beans.

4.3. Physical Methods

Several approaches have been developed to detoxify OTA, including the use of organic and inorganic adsorbents, irradiation, high-pressure, ultrasound, ozone, and biodegradation and/or biological adsorption. Factors such as nutritional quality, organoleptic properties, safety, availability, and cost-effectiveness are determinant in the implementation of these technologies. However, it is out of the scope of the present review to describe the different methods to detoxify OTA in general foods since it is focused in the coffee matrix, but for those readers who are interested in this aspect we recommend other reviews published elsewhere [93–95]. In fact, in coffee, the number of reports on OTA detoxification is very scarce (Figure 2).

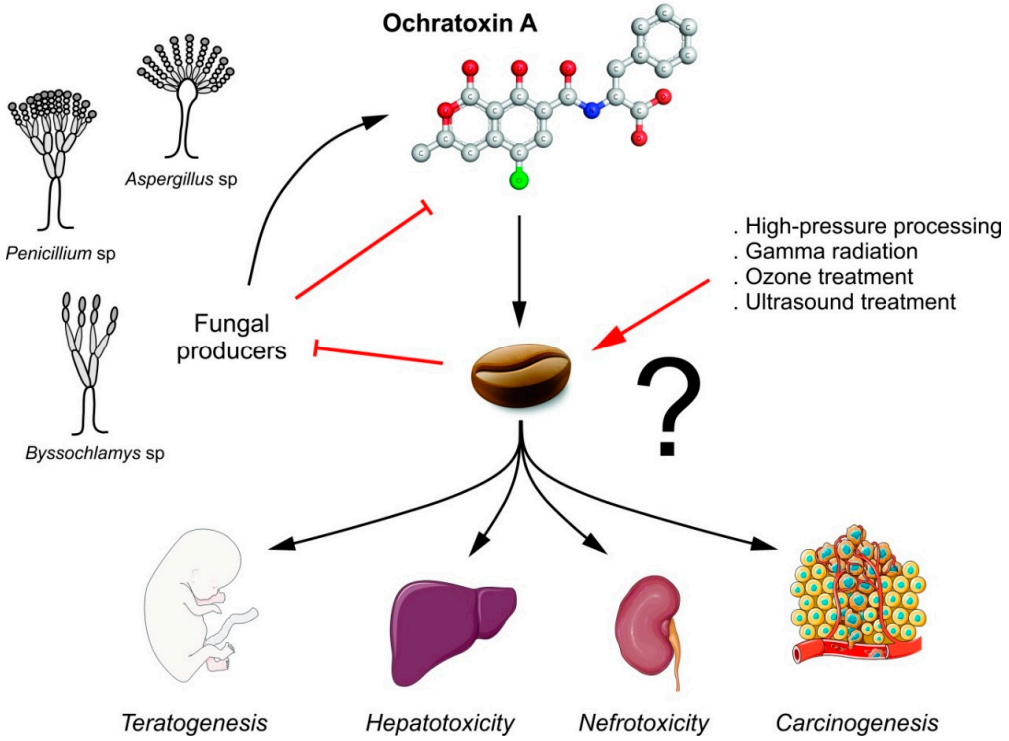


Figure 2. Ochratoxin A in coffee, including its natural sources derived from fungal contamination of beans, their possible toxic effects in humans (black lines) and the potential treatments for minimizing its effects by reducing the fungal contamination of coffee beans (red lines).

One of these examples investigated ozone and ultrasound as alternative processes in the treatment of fermented coffee [96]. The results showed that ozone and ultrasound exhibits a potential in reducing OTA in coffee, due to filamentous fungi inactivation. Besides the biological effect, both treatments reduced the total soluble solids and increased the phenolic compounds content, increasing fruit astringency which concentration is inversely related with brew quality. Meanwhile, this was not observed in sensorial analysis since no perceptible changes in the quality of the beverage were detected. According to these researchers, ozone and ultrasounds could be applied as pre-treatments before coffee fermentation, as they minimally affect the quality of coffee naturally contaminated with OTA. Nutrient

losses or changes are negligible compared to the benefits, improving food safety of the fermented coffee [96].

Recently, the detoxification of OTA using gamma radiation and high-pressure processing (HPP) were proposed, taking into account not only the feasibility and efficacy of the method but also because it overall not promote coffee organoleptic changes [84,93]. In fact, both approaches are known to keep foods' original freshness, but efficacy depends on food composition, particularly water activity. In the case of gamma radiation processing, the study performed by Kumar et al. (2012) showed that green coffee beans having aw of 0.93 when submitted to a dose of 10 kGy OTA was almost completely degraded; while aw of 0.82, 0.65 and 0.59 resulted in approximately 90%, 9% and 5% of OTA reduction, respectively, for an initial concentration of 50 ppb [84]. The influence of HPP in the inhibition of *Aspergillus fresenii* growth was study by Chen et al. (2018), indicating that HPP at pressures of 400 MPa or higher inhibited fungal growth on coffee beans; although no effect was observed on OTA its accumulation was prevented. Additionally, it was observed that 400 MPa not only maintained OTA levels, but also increase γ-aminobutyric acid content after 50 days of storage improving the nutritional value of coffee [85]. Therefore, HPP seems to be a promising technology not to destroy OTA but indirectly avoiding its increment in coffee beans.

Despite the consensus regarding to flexibility and promising results of these new technologies in the treatment of coffee, there is a great concern on definition of optimum treatment conditions (well-directed standard operating procedures in approved laboratories) and toxicity of degradation products formed, and consequent implications on human and animal health.

5. Conclusions

Coffee that is more than a mere beverage, due to its functional and sensorial properties, it could represent a threat, especially to the higher coffee consumer, attending to OTA levels. Good manufacturing practices and hygiene standards during coffee production is strongly recommended to reduce the risk of contamination processed coffee. Additionally, it is necessary to invest in knowledge in order to develop and/or improve effective and friendly technologies to detoxify coffee beans. The inclusion of gamma radiation in the postharvest processing chain of green coffee beans could be a promising approach in order to not only eliminate OTA producer fungi but also the toxicity of performed OTA. Ozone and ultrasound could be applied as pre-treatments before coffee fermentation, while HPP could be introduced in steps presenting high risk of microbiological hazards in the coffee industry without altering the manufacturing process. These promising methods could be complementarily applied toward reducing OTA contamination in the coffee without affecting the sensorial attributes that makes coffee one of the most pleasant products, promoting food safety and quality. However, further studies on emerging new technologies are needed not only in what concerns to improvement and control but also in their application in the coffee treatment, ensuring their effectiveness and safety use in the coffee processing.

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