

Review Article

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Comprehensive review on Ochratoxin A: Occurrence, toxicity, legislation, and Analysis

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Abstract

Food and crops contamination to mycotoxin is a serious concern. Food and Agricultural Organization (FAO) estimated that proximately, 25% of the world's agricultural commodities are contaminated with mycotoxins. Ochratoxin A (OTA) is a toxic mycotoxin produced by some mold species, mainly by some species of *Aspergillus* and *Penicillium*, particularly *A. ochraceus* and *P. verrucosum*, as secondary metabolite. OTA has been detected in different type of food including, cereals, dried fruits, nuts, spices, tea, coffee, and feed. Regard to adverse effect of OTA on human health, some international and national regulatory bodies determine the maximum permitted levels of OTA in foods.

A reliable risk assessment of OTA contamination for humans and animals basically depends on its identification and accurate quantification in food and feed. Therefore, it is important to implement accurate, fast, and cost-effective method for determination of OTA in food products. Chromatographic methods, such as mass spectrometric methods, like gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS) have shown that they can present reliable results.

This review aims to bring up to date the current global status of OTA and presents recent development on different aspect of OTA including sources of exposure, chemical and physical properties, occurrence, health aspect, and determination methods. This includes also the investigation of some of its metabolites and degradation products.

Keywords mycotoxin, Ochratoxin A, dietary exposure, determination, health aspect

1. Introduction

Contamination of food and crops to mold is widespread throughout the world, the Food and Agriculture Organization (FAO) has estimated that approximately 25% of food crops are affected, and destroyed by fungus every year [1]. Fungus contamination and subsequently contamination to mycotoxins is quite possible at pre and/or post-harvest stages of food production and storage.

Mycotoxins contamination, one of the serious problems involving agricultural products, is very critical from the economics, food safety and human health perspectives [2]. While numerous mycotoxins have been identified, about 20 families are harmful to human and animal health. Among these, six families are more common in food commodities, including aflatoxins (AFs), ochratoxins (OTs), fumonisins (FBs), deoxynivalenol (DON),



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zearalenone (ZEA), and patulin [3].

Ochratoxins family, contains more than 20 subtypes, which among them, OTA has attracted the most attention due to its serious toxicity. There are numerous published paper and trustable data reported occurrence of OTA in a wide range of food products such as, cereal, vegetables, nuts, spices, fruits, and coffee in different levels [4].

OTA is a chemically stable compound; therefore, it tolerates ordinary food processing such as heating, freezing, and other common food and production process. There are reports from many countries in the world describing high frequencies of human milk and blood contamination by OTA (although at low levels) however, OTA contamination, in humans, originates from contaminated foods. Moreover, OTA has been shown to be toxic and carcinogenic in animals, as has been classified as group 2B by International Agency for Research on Cancer (IARC). Given the negative effects of OTA on human health, including its association with the Balkan endemic, many countries and national and international organizations have determined permissible limits for OTA use in food, beverages, and animal feed [5].

To ensure food safety, and monitor OTA occurrence, it is imperative to implement technically feasible, accurate, fast, and cost-effective method for determination of OTA in food products. In general, OTA can be determined by two different types of methods: immunologic and chromatographic. Immunochemical assays are based on the ELISA (enzyme-linked immuno-sorbent assay) principle. The chromatographic method includes thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), and ultra-pressure liquid chromatography (UPLC) which may couple to different detectors. Chromatographic methods involve a multiple-step process. However, HPLC with a fluorescence detector (FD) is more common method used due to the strong native fluorescence activity of OTA and the high sensitivity of the method. Common extraction methods are based on the solubility of OTA in organic solvents [6].

The current paper reviews recent development on different aspect of OTA including sources of exposure, chemical and physical properties, occurrence, health aspect, and determination methods. A total of 76 papers were studied. The literature for this review was sourced from the core collection of the Google Scholar online database, covering publications from 2000 to 2025.

Ochratoxins

Sources of exposure

Ochratoxins, a family of mycotoxins produced by several *Aspergillus* and *Penicillium* species, contains more than 20 subtypes, including ochratoxin A (OTA), ochratoxin

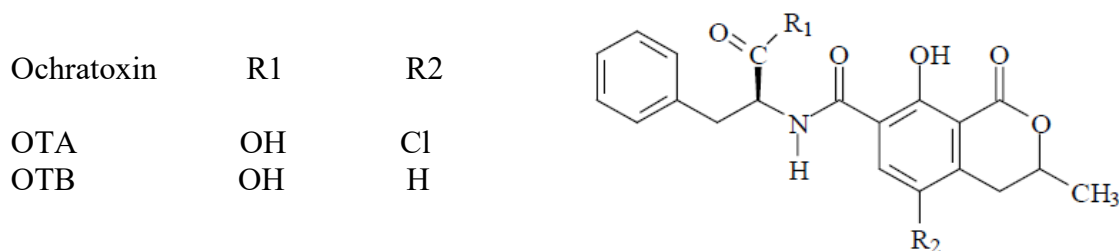
B (OTB), ochratoxin C (OTC), non-amide ochratoxin α (OT α) of OTA and OTC, non-amide ochratoxin β (OT β) of OTB, and hydroxylated OTA and OTB (4R-OH OTA, 4S-OH OTA, 4-OH OTB, 10-OH OTA) [7].

Three generally recognized ochratoxins are designated OTA, OTB and OTC. They were initially described in *Aspergillus ochraceus*. More *Aspergillus* species were shown to produce ochratoxins such as: *A. melleus* and *A. sulphureus*, [8] *A. alliaceus*, *A. sclerotiorum*, *A. ostianus* and *A. petrakii*; *A. niger* var. *niger*; *A. albertensis*; *A. auricomus*; *A. glaucus*, *A. lanosus* and subsequently in other species of *Aspergillus* section *Nigri*: *A. awamori*, and *A. carbonarius* and recently, in two newly described species from coffee, *A. lacticoffeatus* and *A. sclerotio-niger* have been reported by Samson and co-workers [9]. In *Penicillium*, OTA was first detected in *P. viridicatum*. However, later it was reassigned the ochratoxin-producing isolates to *P. verrucosum*; in its current circumscription, *P. viridicatum* does not produce OTA. *P. chrysogenum* and *P. nordicum* have also been reported to produce OTA. In summary, OTA production is currently known in four sections of *Aspergillus* and *Penicillium*, a curiously disjunction taxonomic distribution. As a result, it is unclear in some cases which fungi are responsible for OTA contamination of food. OTA production in *A. niger* is alarming because this species is used in fermentation process which is used for food and enzyme production; industrial strains should be tested for OTA production [10].

Chemical and Physical Properties of OTA

Between several analogues of ochratoxins, OTA is the major compound found as a natural contaminant in plant material. There are small differences in ochratoxins' chemical structure which has marked effects on their respective toxic potentials. OTA is chlorinated and is the most toxic, followed by OTB and OTC. The chemical structure of OTA (Fig. 1) contains a 7-carboxy-5-chloro-8-hydroxy-3, 4 dihydro- (3R)-methyl isocoumarin linked through the carboxyl group to L- β -phenylalanine [11]. OTA, the white crystalline powder, is recrystallized from xylene. It forms crystals that emit green (acid solution) and blue (alkaline solution) fluorescence in ultraviolet light [12]. The melting point of these crystals is 169° C, although partial degradation occurs even at 250 °C [13]. It is highly soluble in polar organic solvents, very slightly soluble in water. In acid hydrolysis, OTA converts to phenylalanine and an optically active lactone acid named ochratoxin α . Sodium salt of OTA is soluble in water. OTA is unstable to light and air, degrading and fading even after brief exposure to light, especially under humid conditions [14].

OTA is a moderately stable molecule and is able to toler-



Ochratoxin		Molecular formula	Molecular mass (g.mol ⁻¹)	CAS number
OTA	R1=H, R2=Cl	C ₂₀ H ₁₈ ClNO ₆	403.8	303-47-9
OTB	R1=H, R2=H	C ₂₀ H ₁₉ NO ₆	369.4	4825-86-9
OTC	R1=C ₂ H ₅ , R2=Cl	C ₂₂ H ₂₂ ClNO ₆	431.9	4865-85-4

Fig 1. Chemical structure of OTA, OTB and OTC [15].

ate the usual food industries processing such as boiling, baking, roasting or fermentation to some extent, therefore it may occur in consumer products. However the degree to which it is destroyed will further depend on other parameters such as pH, temperature and the other ingredients present. OTA is fairly stable to heat; in cereal products, up to 35% of the toxin survives autoclaving for up to 3 hours. Only partly destruction have been reported during cooking and bread making [16].

Baking and roasting have been reported to reduce the toxin content by a mere 20%. In general, thermal food processing methods have received less attention for reducing OTA and other myco-toxins, including AFs, and fumonisins, despite their crucial role in food safety [17]. OTA can be stored in ethanol for at least one year under refrigeration and protected from light, as photolysis may occur on exposure to fluorescent light. It has been reported that OTA solutions in methanol stored at -20 °C are stable over a period of some years.

Natural Occurrence of OTA in Food

OTA can contaminate a wide variety of foods especially agricultural commodities as a result of fungal growth in crops, in the field, during growth, at harvest, post-harvest in storage, and in shipment under favorable environmental conditions especially when they are not properly dried. The molds produce OTA, require specific conditions, such as water activity (aw) ≥ 0.8 , and favorable temperature to propagate and produce OTA [16].

OTA may be present in feed or food products even when the visible mold is not seen. It has been detected in foodstuff such as cereal products, coffee, wine, beer, spices, grape/grapefruit juice, and licorice [18]. Some published studies on natural occurrence of OTA in food and feed

from various countries have been summarized in Table2. OTA can be absorbed from contaminated feed by mono-gastric animals such as pigs, where it is accumulated in the blood and kidneys, and therefore it can be found in products made from them, such as black pudding, sausages, etc. Moreover, it has been detected in milk, cheese and other animal products. Investigations of the frequency and levels of occurrence of OTA in food and human blood samples indicate that foodstuffs are frequently contaminated [19].

Natural Occurrence of OTA in Human Blood and Milk

Human exposure to OTA has been clearly demonstrated by its detection in human blood, urine and breast milk. Investigations in a number of countries have shown that OTA can be found in human blood; in Germany, 57% of 306 samples of human serum tested were positive for OTA, in Sweden 13% of 297 samples, in Canada 40% of 159 samples, in Switzerland 100% of 368 samples showed OTA contamination. In a research done in Portugal, higher OTA levels were observed in the rural populations compared with the urban populations of the zones studied [42].

Human breast milk is the optimal source of nutrition for newborns, but the potential transfer of contaminants like mycotoxins, particularly OTA, from maternal blood to milk is a serious concern. A systematic review aims to provide a comprehensive analysis of global OTA levels in human breast milk and assess the associated health risks was done by Ahmad and coworkers [43]. They studied a total of 39 paper met the inclusion criteria for their review. Results showed that OTA levels compared to limits, estimated infant intake at various ages and health risks assessed using Margin of Exposures (MOEs) and Hazard

Table 1. Natural occurrence of OTA in food and feed from various countries

Sample	No.of positive/ %	Range ng/g	Country	Refrence
Coffee	81% of 82	0.17-6.22	Brazil	[20]
Ginger	29% of 39	1-10	America	[21]
Ginseng	10% of 4	0.4 – 1.8		
Olive oil	80%	> 17.0	Mediterranean	[22]
Red wine	9% of 29	0.1-1.33	Brazil	[23]
Grape juice	0% of 38	-		
red wine	18% of 34	0.03-0.32		
Retail ground	24% of 25	0.27-4.07	Turkey	[24]
Sultanas from vineyard and packaging house	9.8% 58%	> 10 0.026-10	Turkey	[25]
Green coffee	100%	>0.6 , mean=6.7	Different origin	[26]
Cocoa powder	9% of 18	< 0.01 0.22- 0.77	Italy	[27]
Maize grain	7% of 15	0.55-1.40	Croatia	[28]
Peanut seeds	32% of 47	0.5-170	Cordoba- Argentina	[29]
Sorghum, Groun nut Rice bran Sunflower seed Millet	9% f 29 1% of 27 1% of 14 1% of 10 2% of 8	> 10 > 10 > 10 > 10 > 10	India	[30]
Olives and olive pasta	2% of 30	1.18 and 1.86	Athen	[31]
Spices,barley,sorghum,	24.3% of 321	0.054-2.11	Ethiopia	[32]
barley,wheatric e,maize,rye,spe lt,oat	12% of 45	0.02-27.10	Spain	[33]

barley,wheatrice,maize,rye,spe lt,oat	6% of 38	0.27-7.97	Portugal	[33]
Maize	100% of 10	0.16-0.86	Cote d, Ivoire	[34]
Rice	100% of 10	0.16-0.92		
Peanut	60% of 10	0.0-0.64		
Black and white pepper	47.5% of 120	0.15 - 13.58	Malaysia	[4]
Dried red chilli	81.25% of 80	0.2–101.2	Malaysia	[2]
Spices	31.5% of 92	0.45- 18.64	Iran	[5]
Raisins	93 % of 40	0.06–11.4	USA	[35]
Barley	6 % of 60	0.16–185.24		
wheat	13-58%	0.17–14.94		
Dried fruits		0.8–99.1	Morocco	[36]
Beer	10.6 % of 84	<0.43–11.25	Portugal	[37]
Rice	2 % of 36	1.9–2.2	Serbia	[38]
alcoholic and non-alcoholic beverage	20 % of 40	0.24–54.76	Spain	[39]
Maize	71 % of 46	2.14–214.0	Pakistan	[40]
Pasteurized milk	25.8 % of 120	>0.049–18.8	China	[41]

quotient (HQ). Their findings revealed the widespread presence of OTA in breast milk across different regions, with notably higher levels in Africa compared to Asia, South America and Europe. The higher concentrations observed in warmer, humid climates suggest that environmental factors significantly influence OTA contamination [43].

The level of OTA in the samples of 87 mothers' milk in the south of Iran was determined using competitive enzyme-linked immunosorbent assay (ELISA). Results showed among 87 human milk samples, 84 (96.6%) samples had positive results for OTA at a mean level of 24.57 ± 13.6 ng/L. 14 (16%) positive samples revealed more than the maximum limit of 40 ng/L for ochratoxin (range, 1.6-60 ng/L). the authors reported that presence of OTA in the milk of mothers denotes a probable consumption of a contaminated foods [44].

In a related study, to help assess infants' exposure to OTA, milk samples from the Maternal-Infant Research on Environmental Chemicals (MIREC), human milk samples were collected (n = 494) and analyzed for OTA levels by HPLC. They found that 390 (79%) test samples were positive for OTA ranging from 4.5 to 192 ng/L. Based on the food consumption questionnaires distribut-

ed among participants, higher OTA levels were observed with higher consumption of cottage cheese, hot cereal, and whole-grain bread. The mean OTA level in the analyzed milk test samples was well below the amount found in infant formulas sold in Canada, which was determined by Health Canada to be safe [45].

Sixty urine samples of healthy individuals from Coimbra city, Portugal, were investigated, the presence of OTA was found in 42 samples, at concentrations above the LOQ, ranged between 0.021 and 0.105 ng/ ml [30]. It must be considered that as a consequence of the estimated relatively long half- life of OTA in human blood of 20-50 days (strong binding to serum proteins; probable entero-hepatic circulation), the OTA concentration in the blood should be higher than in the daily diet.

Health aspects of OTA

Based on IARC report, OTA is nephrotoxic, mutagenic, carcinogenic, teratogenic, and immunosuppressive in a variety of animal species can be absorbed from the gastrointestinal tract and stomach. Ochratoxin absorption also can takes place in the small intestine. In non-ruminant species such as pigs, chickens, rabbits, and rats, around half of the ingested OTA may be absorbed and distributed

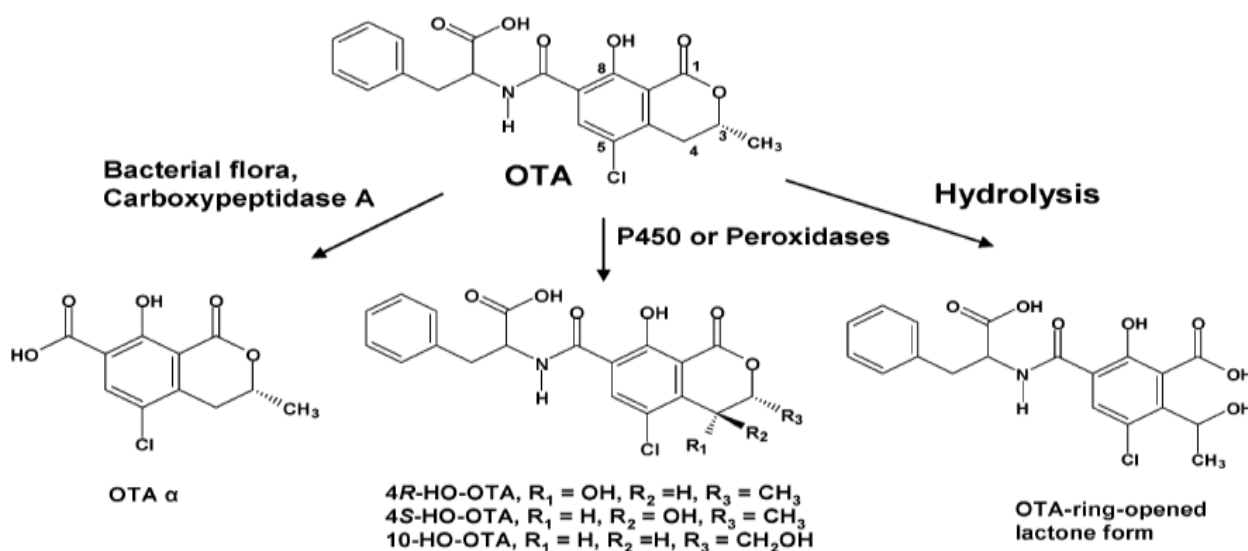


Fig 2. Major metabolites of ochratoxin A [47].

via blood, mainly to the kidneys, and at lower concentrations to the liver, muscle and fat. Transfer of OTA to the milk has been demonstrated in rat, rabbit and human. In contrast, little of this toxin is transferred to the milk of ruminants. It has been reported in ruminant species such as the cow, OTA can be hydrolyzed to non-toxic compound due to metabolism of the ruminant protozoa [46].

Two known metabolism of OTA are include production of OTα and hydroxylated derivatives (Fig. 2). OTA is cleaved into phenylalanine and OTα by peptidases in the intestinal mucosa, and in other organs such as pancreas as well as in vitro. OTα was previously regarded as harmless, but evidence for its genotoxicity has been recently found. In the presence of NADPH, liver microsomes from pigs, rats, and humans metabolize OTA to produce hydroxylated derivatives (4R)- and (4S)-4- hydroxyl OTA [46].

OTA has an established cancerogenic effect on animals and a supposed cancerogenic effect on humans. However, no adequate human studies of the relationship between exposure to OTA and human cancer have been reported so it has been classified by the International Agency for Research on Cancer as group 2B [14]. The available evidence shows that OTA is a genotoxic carcinogen by induction of oxidative DNA lesions coupled with direct DNA adducts by quinone formation. This mechanism of action should be used to establish acceptable intake levels of OTA from human food sources [48].

Liu reported the OTA toxicokinetics profile is related to its low water solubility and high protein-binding affinity (approximately 99.98% binding to plasma proteins, mainly albumin), contributing to its extended half-life

of 35 days. This facilitates OTA accumulation in major organs such as kidneys, liver, brain, and adipose tissues, enabling enterohepatic recirculation, which prolongs its toxicity [49, 16]. It has been shown that the generation of reactive oxygen species (ROS) is the major pathway in OTA-induced toxicity, particularly targeting kidney proximal tubule cells [50].

OTA is known as Balkan endemic nephropathy. A relatively high frequency of contamination of cereals and bread with OTA has been reported in an area of Yugoslavia where Balkan endemic nephropathy is present. There are some evidences demonstrated after exposure to OTA, it can make some problem for kidney including functional and morphological changes, but other effects have been observed as well for example, liver and heart morphology abnormalities, blood clotting, reduced egg production, gastrointestinal/ renal and lymphoid tissue lesions. Oral LD50 for animal being estimated to range between 1.0 ng/kg for pigs, 0.2 for dogs and 4.7 ng/ kg for tronts [47].

International Legislation of Ochratoxin A

Recently, both monitoring programs and research have been conducted, aimed at individuating the status of world-wide mycotoxin contamination and at finding the critical control points in the food chain. However despite scientific and technical knowledge on mycotoxin management, and improvements in techniques used for production and storage of agricultural commodities and crops, it is not possible to prevent the growth of molds and consequently elimination of OTA from food cannot be done easily. Therefore limits which are as low as rea-

Table 3. The European Union limits for OTA [18]

Category	Maximum Level (µg/kg)	Remarks
Dried fruits		
Dried vine fruits (currants, raisins and sultanas) and dried figs	8.0	
Other dried fruits	2.0	
Date syrup	15	
Pistachios		
Pistachios to be subjected to sorting or other physical treatment before placing on the market for final consumer or use as an ingredient in food	10.0	If tree nuts 'in shell' are analysed, it is assumed that all the contamination is on the edible part.
Pistachios placed on the market for final consumer or use as ingredient in foods	5.0	Same remark as above.
Dried herbs and roots		
Dried herbs	10.0	
Ginger roots (dried) for use in herbal infusions	15	
Marshmallow roots (dried), dandelion roots (dried) and orange blossoms (dried) for use in herbal infusions or in coffee substitutes	20	
Seeds and soybeans		
Sunflower seeds, pumpkin seeds, (water) melon seeds, hempseeds, soybeans	5.0	
Cereals and cereal products		
Unprocessed cereal grains	5.0	Applies to unprocessed cereal grains placed on the market before first-stage processing.
Products derived from unprocessed cereal grains and cereals placed on the market for the final consumer (except specific exclusions)	3.0	Includes processed cereal products. Products contain at least 80 % cereal products.
Bakery wares, cereal snacks and breakfast cereals		
Products not containing oilseeds, nuts or dried fruits	2.0	
Products containing at least 20 % dried vine fruits and/or dried figs	4.0	
Other products containing oilseeds, nuts and/or dried fruits	3.0	
Non-alcoholic malt beverages	3.0	
Wheat gluten not placed on the market for the final consumer	8.0	
Coffee and cocoa		
Roasted coffee beans and ground roasted coffee (except soluble coffee)	3.0	
Soluble coffee (instant coffee)	5.0	
Cocoa powder	3.0	
Dried spices		
Dried spices except <i>Capsicum</i> spp.	15	Applies also to mixtures of dried spices.
<i>Capsicum</i> spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne or paprika)	20	

Liquorice (Glycyrrhiza species)		
Liquorice root (dried), including as an ingredient in herbal infusions	20	
Liquorice extract for use in food (particularly beverages and confectionery)	80	Applies to pure undiluted extract (1 kg extract from 3–4 kg root).
Liquorice confectionery containing $\geq 97\%$ liquorice extract on dry basis	50	
Other liquorice confectionery	10.0	
Wine, fruit wine, grape products		
Wine and fruit wine (including semi-sparkling and sparkling, excluding liqueur wine and wine with $\geq 15\%$ vol alcohol)	2.0	Applies to products from the 2005 harvest onwards.
Aromatised wine, aromatised wine-based drinks and aromatised wine-product cocktails	2.0	Applies to products from the 2005 harvest onwards; level depends on wine/grape must proportion.
Grape juice, grape juice from concentrate, concentrated grape juice, grape nectar, grape must and concentrated grape must (for final consumer)	2.0	For concentrates, level applies to reconstituted product; applies to products from the 2005 harvest onwards.
Baby foods and special medical purposes		
Baby food and processed cereal-based food for infants and young children	0.50	Applies to the dry matter of the product.
Food for special medical purposes intended for infants and young children	0.50	For milk-based products: ready-to-use product (or reconstituted); for others: dry matter.

sonably achievable should be set [19, 74]. Codex established maximum limit for OTA be at a level of 5 μg / kg for Wheat (including raw common wheat, raw durum wheat, raw spelt and raw emmer), barley, and rye.

The European Union initially set maximum allowable limits for ochratoxin in food products in 2002, thereafter updating them each year. In accordance with the latest edition of the EU regulatory framework (2023), the permissible limits for ochratoxin A across different food categories are specified in the table below. (Table 3).

Determination of OTA

A review of current techniques for mycotoxin analysis indicated that an analytical procedure can be devised using different approaches. Results of surveys indicated that there are a significant number of methods that can be used or modified to satisfy certain analytical requirements however there is no best technique for mycotoxin analysis. The analyst's preference, the sample matrix, the target mycotoxin, and the availability of supplies and equipment, must be taken into consideration when choosing a procedure for the analysis [6].

In general determination of OTA is not easy due to its being found in complex matrixes and should be detected in low concentrations. Methods for determining OTA in agricultural commodities and food products have been verified by AOAC International and by various international

committees [51-54]. Generally, all chemical analytical methods contain the basic steps as following: sampling, sample preparation, extraction, clean-up, detection and quantification.

Many methods for quantifying OTA have been developed. OTA can be determined by two different types of methods: immunologic and chromatographic. Immunochemical assays such as enzyme-linked immuno-sorbent assay principle (ELISA), and fluorescence polarization immunoassay [41]. The chromatographic method includes thin-layer chromatography (TLC) and high performance liquid chromatography coupled with fluorescence detection (HPLC-FD), liquid chromatography mass spectrometry (LC-MS/MS) which involves a multiple-step process. Based on Yang and co-workers the HPLC-FLD is the most well-known method for detecting OTA because of its wide availability, lower cost than that of mass spectrometry, high sensitivity (OTA molecules are strongly fluorescent), simple operation, and satisfactory accuracy [6, 41].

Sampling and Sample Preparation

The first step encountered with mycotoxin analysis is the selection of a representative sample. The production of mycotoxins is not homogeneous; this problem is most severe with grain, nuts, and oilseed commodities. As an example OTA contamination often occurs in "spots" during

storage in silos therefore a poor sampling procedure of the batch can miss highly infected grains. The examination of raw coffee for OTA in conjunction with the German Food Monitoring Program of 2000 was undertaken with various sample weights (5, 10 and 30 kg) in order to identify the influence of the sample weight on the result. Overall, the sample weight did not have an effect on the detection and level of the OTA content in the samples of raw coffee examined [55].

Extraction

In general, food and feed samples can't be analyzed without some preliminary sample preparation, because mycotoxin especially OTA is usually present in food at very low concentration level and the food or feed matrix are complicated. Therefore, sample extraction is often a critical and complex part in the overall analytical process, since different matrix need different strategies [56].

Common extraction methods are based on solubility of the mycotoxin in organic solvents. A suitable solvent can increase extraction efficiency. Selection of solvent depends on the type of mycotoxin and chemical specification of sample matrix. Extraction is usually performed with a mixture of water and polar organic solvents, such as methanol, acetone or acetonitrile, depending on the type of matrix. Methanol and water in different ratio is the most popular solvent applied for determination of mycotoxins in food [57]. The other solvent namely pure water, acetonitrile/water, chloroform/phosphoric acid, dichloromethane, and phosphate buffer saline (PBS) [58] has been used. Whereas extraction with chloroform used in a number of previous methods, has largely been superseded by these other solvents as part of the international drive to reduce the consumption of chlorinated solvents. Generally one solvent system that gives a good recovery with one matrix may not be suitable or is less efficient with other sample matrix. As an example, methanol: water (80:20, v/v) with solution of 1% NaHCO_3 appeared to be the most useful extraction solvent for determination of OTA in coriander, ginger, paprika and black pepper depending on kind of matrix but in case of cloves only, none of the methods of extraction was appropriate [59].

The extraction solvent itself has also received closer attention in recent years. The amounts of AFB_1 and B_2 extracted from naturally contaminated raw peanuts using the water slurry modification of AOAC Method 970.45 showed to be a function of both methanol concentration and solvent-peanut ratio. The optimum combination resulted in extraction of 12% more AFs than AOAC Method 970.45. Among other factors, cost, toxicity, and disposal of solvents must now be considered before selecting an extraction solvent for any mycotoxin method.

Clean up

The third step of mycotoxin analysis is clean up of the sample extract, in order to eliminate fat and other food components which interfere with the detection of mycotoxins. The most common clean-up methods are liquid-liquid extraction, solid phase extraction, multi-functional cleanup column and immunoaffinity column (Fig. 3).

Liquid-liquid extraction also called solvent extraction, is defined as a process that involves the separation of two or more components due to their unequal solubility into liquid phases, dispersion created during the mixing is then separated by gravity or by centrifugal force. This method is popular in determination of AFs and OTA in different samples, and had been used for pig kidneys and muscles, cereal and feed reported that liquid-liquid extraction is rapid, inexpensive and reliable for wheat and oat, but not for barley, rye and mixed cereal matrices such as trout feed [56].

Solid phase extraction (SPE) has become widely used in recent years as a rapid and solvent efficient cleanup technique. The most commonly used SPE columns and cartridges contain silica gel, Florisil, phenyl, aminopropyl, or strong anion exchange (quaternary ammonium) bonded phases. European Committee for Standardization (CEN) has adopted two LC methods for determination of OTA in cereals and cereal products by using silica gel or C18 cartridges as clean-up method [6].

A recent development in small column technology is the multi-functional cleanup column, which provides a rapid removal of interferences from food extracts by lipophilic and charged sites in the proprietary packing material, in which mycotoxins pass through the column with excellent recoveries. Using multi-functional column is a rapid (within 2 h), simple and reproducible for the analyses of AFs in spices. This method can be used to analyze mycotoxins more rapidly and safely compare to the methods using a chloroform extraction step [60].

The most important development in the field of clean-up methods during the last few years is Immunoaffinity columns (IACs). IACs consist of an anti-mycotoxin antibody bound to a gel material contained in a small plastic cartridge. By forced passing the crude extract through the column, mycotoxins are left bound to the antibody. The analyte is eluted and extraneous material is washed off by water or aqueous buffer, and the toxin is eluted with an appropriate solvent like acetonitrile, methanol or methanol-buffer. IACs are commercially available and have been routinely employed for determining OTA in different food and feed [56].

These columns have the advantages of speed and simplicity compared to conventional clean-up and of high specificity thereby producing extracts free of interferences.

The major advantage of IACs is the ability of applying the same procedure to differing matrices, achieve high recoveries and to obtain chromatograms without interferences of unknown picks. A disadvantage can be the need to push the sample extract through the column at a slow but steady rate, which is boring and time consuming when carried out manually and can be a source of variable recoveries when not properly controlled [56].

Other sample pretreatment technique include quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction; solid bar microextraction (SBME), immunoaffinity magnetic beads, molecularly imprinted polymers, and aptamer affinity columns [61].

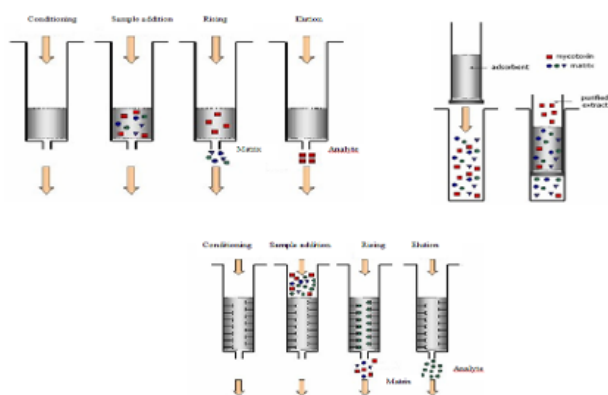


Fig 3. Clean-up methods: (a) solid phase extraction; (b) Multifunctional; (c) Immunoaffinity column.

Detection

Mycotoxin detection is performed by two groups of methods: 1- chromatographic methods such as: TLC, LC, and in few cases GC. 2- Immunochemical methods such as ELISA (Enzyme linked immunosorbent assay). Traditional chromatographic techniques such as thin layer chromatography (TLC), liquid chromatography (LC), UPLC, and gas chromatography (GC) are widely employed for detection and quantitation of the mycotoxins. The ELISA method also is most commonly used. All TLC methods and most of the LC methods used for AFs and OTA separation and quantitation, the toxin concentration are based on the intensity of the fluorescence observed. Chromatographic methods for mycotoxins were reviewed by Rahmani with coverage of a wide range of mycotoxins [62].

High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) in recent years has been one of the most popular methods for determination OTA in food [63]. The important advantag-

es of HPLC that make it more popular, comparing with other methods, include ability to apply for poorly volatile, nonvolatile, polar and ionic compounds; Increasing sensitivity; accuracy and precision of the method; variety of detection system available and low detection limit [41].

Separation of the mycotoxin has been performed for many years by normal and reversed phase column HPLC. However the vast majority of separations are performed on reversed-phase systems with mobile phases composed of water, methanol and acetonitrile mixtures. The optimum mobile phase was found to be a ternary mixture of the three solvents, tailored to the individual characteristics of the HPLC column [64].

Recent research on OTA determination by HPLC has focused mainly on selective detection systems such as diode array, UV, FD, and mass spectrometry (MS) and on incorporation of LC into automated methods [58]. The reversed-phase HPLC–UV technique was an early method used for the determination of mycotoxins in grains, which was based on applying an acidic mobile phase with phosphoric acid. However, the retention times were highly variable. In sequence, a general method for mycotoxin analysis was developed, based on HPLC with an alkyl phenone retention index and photodiode-array (PDA) detection in 2 different eluents. HPLC with fluorescence detection (FD) becomes the method of choice because of the available short and high-resolution columns and of the sensitivity of fluorescence detectors and its potential for automation [65].

Since OTA shows fluorescence under UV light, spectrofluorimetric detection has been adopted and HPLC with FD as the final separation and determination method has become the widely used method in the last 20 years [64].

Thin Layer Chromatography (TLC)

TLC was an extremely powerful, rapid, and low-cost separation technique in mycotoxicology before HPLC techniques became popular. Since 1990, it has been considered as an AOAC official method and the method to identify and quantify AFs at levels as low as 1 ng/g. According to reports and articles, mycotoxins were easily separated by TLC using several solvents [66].

Although the number of publications on TLC has declined in recent years, TLC method remains a method of choice, on occasion, for example measurement of ochratoxin in different samples such as green coffee [67]. However, with this technique, the procedure is variable resulting to a large coefficient of variation and poor precision. The main disadvantage associated with TLC is the lack of precision.

The over pressured-layer chromatography (OPLC) method has the advantages of the HPTLC and HPLC methods.

The linear OPLC is a forced flow technique, using external pressure on chromatoplate sealed on the edges and a pump system for the admission of mobile phase into the stationary phase. Comparing with the HPLC, it requires less mobile phase, using off-line method, and allows faster examination with the possibility of parallel analysis. OPLC is more efficient than TLC, providing better resolution and more compact spots [68].

Chromatography and mass spectrometry

The UPLC coupled mass spectrometry (MS) known as LCMS, provides a great opportunity for the analysis of mycotoxins such as OTA, especially because of elimination the need for sample derivatization for fluorescence activity enhancement. Furthermore, LC tandem mass spectrometry (LC/MS/MS) is an extremely sensitive and accurate technology for OTA analysis. Several researchers discussed the use of LC/MS/MS in OTA detection in various food and feed samples. However, regard to the high cost of the equipment and complexity of the test method, only few studies have been published for OTA determination by LCMSMS [74].

Based on Rahmani and coworkers [62], before mass spectrometer, UPLC will separate the sample to chemical compounds. Then mass spectrometer will ionize molecules and sort, and identify them according to their mass-to-charge ratio (m/z).

In a related study, LC MSMS was compared with HPLC-FD for determination of OTA in liquorice extract powder. The results indicate that the LC-MS/MS method was more specific and sensitive for the analysis and confirmation of OTA than HPLC method because of false positive in HPLC method in some sample [69].

A LC-MS/MS was used for the simultaneous determination of aflatoxins, OTA, zearalenone, deoxynivalenol, toxins T2, HT2 and fumonisins in cereals. The application of the method on 30 grain samples showed that the method has sufficient accuracy to be used as a routine analysis method for small levels of mycotoxins [70].

Zhang reported that due to the simple sample preparation and high sensitivity and selectivity, the LC-MS/MS appears to be the most promising tool and has been used for the in-vivo detection of OTA in rats, chickens, and pigs [71]. Although, LCMSMS is expensive equipment and the cost of its repair and maintenance is very high, especially in developing countries. In addition, the skill of the user is also very important in using this device.

Enzyme Linked Immunosorbent Assay (ELISA)

ELISA technique involves either coating of a test antigen to the surface, or coating of the antibody to the surface. ELISA can be used for determination of mycotoxin di-

rectly or indirectly. In directly method ELISA technique involves attachment of the antibody to the surface, and competition between the toxin and a toxin-labeled enzyme for the limited amount of antibody. This technique is widely known as “direct ELISA” and is the most widely used in commercial mycotoxin ELISAs. If the mycotoxin-specific antibody is labeled with an enzyme, or a secondary antibody (labeled with enzyme) is used, this type of technique is widely known as an “indirect ELISA” [72].

ELISA is a widely used method for OTA detection, and a new, highly sensitive OTA ELISA has been developed using monoclonal antibodies. A direct ELISA format showed promise for OTA quantification in medicinal herbs, exhibiting a low IC₅₀ value and strong correlation with HPLC [58]. ELISA has been used for determination of OTA in food. Positive samples were also analyzed and confirmed by HPLC. The results obtained by ELISA were very close to those obtained by HPLC [73, 76].

Conclusion

The current review was aiming to discuss the OTA occurrence in various food and feed, its health aspect, and analytical methods involved in OTA detection and estimation. Ochratoxin is a chemically diverse group of fungal metabolites that showed a wide some toxic effects.

Ochratoxin A (OTA) occurs very frequently and in some cases at levels higher than maximum accepted level and may cause severe human or animal injury. Therefore serious monitoring has to be done by regulatory bodies.

The foregoing review also has highlighted a few of novel techniques and trends in OTA determination, with emphasis on applications to food analysis. Different methods applicable for OTA analyses steps such as extraction, clean up, and detection were discussed. Analysts have to select the methodology most suitable for the particular food and feed matrix and have to introduce the necessary cleanup and concentration steps. However, qualitative and quantitative analysis of OTA has been done, but rapid, sensitive, and cost effective methods needed to be established. Moreover, Sampling remains one of the most areas of concern while, more research is needed.

Although OTA occurrence in food and feed is not a new issue, and it is almost difficult to manage the exposure of toxin producing storage molds in feed and foodstuffs, therefore more efforts to control its exposure is necessary to be programmed by national, and international governing bodies for prevention of OTA exposure.

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Declarations

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Consent for publication

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Competing interests

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