Review Article

Alternaria Mycotoxins in Food and Feed: An Overview

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Received 24 March 2017; Revised 31 July 2017; Accepted 26 September 2017; Published 26 October 2017

Academic Editor: Giuseppe Zeppa

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Alternaria is one of the major mycotoxigenic fungal genera with more than 70 reported metabolites. *Alternaria* mycotoxins showed notably toxicity, such as mutagenicity, carcinogenicity, induction of DNA strand break, sphingolipid metabolism disruption, or inhibition of enzymes activity and photophosphorylation. This review reports on the toxicity, stability, metabolism, current analytical methods, and prevalence of *Alternaria* mycotoxins in food and feed through the most recent published research. Half of the publications were focused on fruits, vegetables, and derived products—mainly tomato and apples—while cereals and cereal by-products represented 38%. The most studied compounds were alternariol, alternariol methyl ether, tentoxin, and tenuazonic acid, but alternuene, altertoxins (I, II, and III), and macrosporin have been gaining importance in recent years. Solid-liquid extraction (50%) with acetonitrile or ethyl acetate was the most common extraction methodology, followed by QuEChERS and dilution-direct injection (both 14%). High- and ultraperformance liquid chromatography coupled with tandem mass spectrometry was the predominant determination technique (80%). The highest levels of alternariol and alternariol methyl ether were found in lentils, oilseeds, tomatoes, carrots, juices, wines, and cereals. Tenuazonic acid highest levels were detected in cereals followed by beer, while alternariol, alternariol methyl ether, tenuazonic acid, and tentoxin were found in legumes, nuts, and oilseeds.

1. Generalities of *Alternaria*

1.1. Introduction. *Alternaria* is a fungal genus that includes saprophytic and pathogenic species and that is widespread in nature. They can infect a wide variety of crops in the field and in the postharvest stage causing considerable losses due to fruit and vegetable decay [1]. The most common *Alternaria* species include *A. alternata*, *A. tenuissima*, *A. arborescens*, *A. radicina*, *A. brassicae*, *A. brassicicola*, and *A. infectoria*. They colonize a range of plants including cereals, tomatoes, apples, grapes, oil crops, oilseeds, sunflower seeds, oranges, lemons, melons, cucumbers, cauliflowers, peppers, and tangerines [2].

The optimum growth temperatures for *Alternaria* range from 22 to 30°C; however, minimum growth temperatures ranging from 2.5 to 6.5°C and, even lower, from 0 to −5°C in cooler regions were reported [3]. Their ubiquitous occurrence and ability to grow and produce toxins even under unfavorable conditions (low temperatures and low water activity)

make the *Alternaria* genus responsible for the spoilage of several commodities during transport and storage, even if they are refrigerated [1]. Therefore, *Alternaria* species has been shown to be a significant contaminant of refrigerated fruits, vegetables, and stored feedstuffs [4]. *A. alternata* is the most important within the genus as regards to mycotoxin contamination of fruits and vegetables. However, the production of host specific toxins from pathogenic *A. alternata* strains seems not to be a real problem in terms of food safety while a much more important problem is its saprotrophic strains, which colonizes harvested plant products and can produce reasonable amounts of certain mycotoxins, which exert poisonous effects after consumption by humans [5].

Alternaria species have the ability to produce more than 70 toxins, which play important roles in fungal pathogenicity and food safety, since some of them are harmful to humans and animals [6]. The studied *Alternaria* secondary metabolites belong to diverse chemical groups such as

nitrogen-containing compounds (amide, cyclopeptides, etc.), steroids, terpenoids, pyranones, quinines, and phenolics [7]. The major *Alternaria* toxins belong to the chemical groups dibenzo- α -pyrones, which include alternariol (AOH) and alternariol monomethyl ether (AME) and cyclic tetrapeptides represented by tentoxin (TEN). These mycotoxins were the most commonly studied metabolites produced by *Alternaria* strains on different substrates (tomato, wheat, blueberries, walnuts, etc.) and some of the main *Alternaria* compounds thought to pose a risk to human and animal health because of their known toxicity and their frequent presence as natural contaminants in food [8].

In the last decade, Ostry [1], Scott et al. [9], and Fernández-Cruz et al. [10] have studied the occurrence of the major *Alternaria* mycotoxins: AOH, AME, and TEN. However, food-relevant *Alternaria* species are able to produce many more metabolites including that known as emerging *Alternaria* mycotoxins described as potentially hazardous.

In this sense, mycotoxin research has focused in recent years on the emerging group, along with the major *Alternaria* toxins [11]. Emerging *Alternaria* mycotoxins mainly belong to five chemical classes: pyranones or benzopyranes (altenuene (ALT); altenuisol (AS); altenuisin (ALN)), amine/amide metabolites (tenuazonic acid (TeA); altersetin (ALS)), perylene-quinones (altertoxins (ATXs), alterperyleneol or alteichin (ALTCH), and stemphylytoxin (STE)), and anthraquinones (Macrosporin A, Altersolanol (As-A)). Table 1 summarizes the main *Alternaria* toxins reported in the scientific literature, for both major and emerging compounds, including the chemical name, molecular formula and weight, and CAS number.

1.2. Toxicity of *Alternaria* Mycotoxins. Toxicological data are limited to the above-mentioned major mycotoxins, and even these data are incomplete, with neither good bioavailability studies nor long-term clinical studies available [12]. Although little is known so far about their properties and toxicological mechanisms, bioavailability, and stability in the digestive tract, *Alternaria* toxins have been shown to have harmful effects in animals, including cytotoxicity, fetotoxicity, and teratogenicity. They have been related to a range of pathologies from hematological disorders to esophageal cancer. They are also mutagenic, clastogenic, and estrogenic in microbial and mammalian cell systems and tumorigenic in rats [1, 13].

The benzopyrone group is the most studied among all the *Alternaria* mycotoxins, and it was the first to be analyzed. This group encompasses the two major toxins AOH and AME, as well as ALT and AS. Although their toxicity is not fully understood and varies from one cell system to another, AOH and AME toxicity have been identified in various *in vitro* and *in vivo* systems [14, 15]. Lehmann et al. [16] reported the AOH estrogenic potential and its inhibitory effects on cell proliferation. Furthermore, AOH induced marked phenotypic changes in mice macrophages, which could not be directly linked to an initial AOH-induced ROS production, cell cycle arrest or autophagy as seen as a consequence of AOH-induced double-stranded DNA breaks [17]. AME and AOH were not very acutely toxic; however, they do exert genotoxic, mutagenic, and carcinogenic properties and

show remarkable cytotoxicity in microbial and mammalian cell culture. Moreover, AOH and AME were able to induce DNA strand break and gene mutations in cultured human and animal cells [18, 19]. They interfered with the activity of human topoisomerases by affecting the stabilization of topoisomerase II-DNA-intermediates and the modulation of the redox balance in human colon carcinoma cells yet without any apparent negative impact on DNA integrity [20]. AOH and AME are frequently found in combination [21] as they share a common biosynthesis pathway [22]. Exposure of HCT116 cells to low AOH and AME concentrations resulted in loss in cell viability throughout the activation of the mitochondrial apoptotic process associated with the opening of the mitochondrial permeability transition pore and the loss of mitochondrial transmembrane potential. Higher toxic potential indicated synergetic effects when applied together [23]. Thus, AOH and AME levels decreased cell viability in Caco-2 cells through being more cytotoxic the binary combination [24]. AOH cytotoxicity decreased in Caco-2 cells in simultaneous combination with Soyasaponin I [25].

Although there have been no *in vivo* genotoxicity or carcinogenicity studies on experimental animals or humans for *Alternaria* toxins, some indications of precancerous changes have been reported in esophageal mice mucosa. Their presence in cereal grain has been suggested to be associated with high levels of human esophageal cancer in China, as well as in areas of Africa where high levels of *Alternaria alternata* contamination have been found [26]. The underlying mechanisms of action have not yet been fully clarified. Limited data are available for long-term toxicity effects of low concentrations of *Alternaria* toxins and their synergistic effect on other contaminants [13]. ALT has shown high acute toxicity with a LD₅₀ value of 50 mg/kg bw in mice, whereas TeA is considered to be the most acutely toxic among the *Alternaria* mycotoxins [27]. TeA is known to occur in high concentrations in commodities and has attracted increasing attention in recent years [11]. It inhibits protein biosynthesis at the ribosomal level in mammalian cells by suppressing the release of newly formed proteins from the ribosomes and is biologically active as it exerts cytotoxic, phytotoxic, antitumor, antiviral, antibiotic, and antibacterial effects [28]. TeA toxicity has been reported in chick embryos and several animal species, including guinea pigs, mice, rabbits, dogs, and rhesus monkeys [29]. TeA is acutely toxic in living organisms and its LD₅₀ value in mice is similar to that of the *Fusarium* mycotoxin deoxynivalenol (DON) [30]. TeA showed acute toxic effects in rodents (oral LD₅₀ for mice, 81–186 mg/kg bw; and for rats, 168–180 mg/kg bw) and chicken embryos (LD₅₀ 0.55 mg/egg). A short-term animal trial (33 days) on monkeys led to vomiting, bloody diarrhea, and hemorrhagic lesions in the intestinal tract after a treatment with 89.6 mg TeA/kg bw per day [31]. Additionally, TeA has been made responsible for the outbreak of onyalay, a human hematologic disorder disease occurring in Africa, and esophageal cancer in Linxian Province, China [29]. There are also reports of the interacting effects of binary combinations of TeA with *Fusarium* toxins (DON, nivalenol, zearalenone, enniatin B, and aurofusarin) in Caco-2 cells, leading to a decrease in cytotoxicity, compared to the expected synergistic effects.

TABLE 1: Chemical name, molecular formula, molecular weight, and CAS number for the main *Alternaria* mycotoxins.

Group and chemical class	Mycotoxin	Chemical name	Molecular formula	Molecular weight (g/mol)	CAS number
<i>Major mycotoxins</i>					
Benzopyrones/pyranones	Alternariol (AOH)	3,7,9-Trihydroxy-1-methyl-6H-dibenzo[b,d]pyran-6-one	C ₁₄ H ₁₀ O ₅	258.226	641-38-3
	Alternariol monomethyl ether (AME)	3,7-Dihydroxy-9-methoxy-1-methyl-6H-dibenzo[b,d]pyran-6-one	C ₁₅ H ₁₂ O ₅	272.253	23452-05-3
Cyclic tetrapeptides	Tentoxin (TEN)	Cyclo[N-methyl-L-alanyl-L-leucyl-(αZ)-α,β-didehydro-N-methylphenylalanyl-glycyl]	C ₂₂ H ₃₀ N ₄ O ₄	414.498	28540-82-1
	Dihydrotentoxin (DH-TEN)	Cyclo-(L-leucyl-N-methyl-(E)-dehydrophenylalanyl-glycyl-N-methyl-L-alanyl)	—	—	—
	Isotentoxin (isoTEN)	Cyclo-(L-leucyl-N-methyl-L-phenylalanyl-glycyl-N-methyl-L-alanyl)	—	—	65452-16-6
<i>Emerging mycotoxins</i>					
Benzopyrones/Pyranones	Altenuene (ALT)	(2S,3S,4aS)-2,3,7-Trihydroxy-9-methoxy-4a-methyl-2,3,4,4a-tetrahydro-6H-benzo[c]chromen-6-one	C ₁₅ H ₁₆ O ₆	292.284	889101-41-1
	Altenuisol (AS)	2,7,9-Trihydroxy-3-methoxybenzo[c]chromen-6-one	C ₁₄ H ₁₀ O ₆	2742.256	42719-66-4
	Altenusin (ALN)	2-(4,5-Dihydroxy-2-methylphenyl)-6-hydroxy-4-methoxybenzoic acid (2E,4E)-5-(4-Methoxy-5-methyl-6-oxopyran-2-yl)-3-methylhexa-2,4-dienoic acid	C ₁₅ H ₁₄ O ₆	29026.806	31186-12-6
	Infectopyrone		C ₁₄ H ₁₆ O ₅	2642.738	—
Amine/amide metabolites	Tenuazonic acid (TeA)	(5S)-3-Acetyl-1,5-dihydro-4-hydroxy-5-[(1S)-1-methylpropyl]-2Hpyrrol-2-one	C ₁₀ H ₁₅ NO ₃	197.231	27778-66-1
	Altersetin (ALS)	2H-Pyrrol-2-one,1,5-dihydro-4-hydroxy-5-(1-hydroxyethyl)-3-(1S,2R,4aS,6R,8aR)-1,2,4a,5,6,7,8,8a	C ₂₄ H ₃₃ NO ₄	399.241	485815-64-3
Perylenequinones	Altertoxin I (ATX I)	(1S,12aR,12bS)-1,2,11,12,12a,12b-Hexahydro-1,4,9,12atetrahydroxy-3,10-perylenedione	C ₂₀ H ₁₆ O ₆	352.337	56258-32-3
	Altertoxin II (ATX II)	(7aR,8aR,8bS,8cR)-7a,8a,8b,8c,9,10-Hexahydro-1,6,8c-trihydroxyperylo[1,2-b]oxirene-7,11-dione	C ₂₀ H ₁₄ O ₆	350.321	56257-59-1
	Altertoxin III (ATX III)	(1aR,1bS,5aR,6aR,6bS,10aR)-1a,1b,5a,6a,6b,10a-Hexahydro-4,9-dihydroxy-perylo[1,2-b:7,8-b']bisoxirene-5,10-dione	C ₂₀ H ₁₂ O ₆	348.306	105579-74-6
	Alterperylene/Alteichin (ALTCH)	(1S,12aR,12bS)-1,4,9,12a-tetrahydroxy-2,12b-dihydro-1H-perylene-3,10-dione	C ₂₀ H ₁₄ O ₆	350.32	88899-62-1
	Stemphytoxin I (STE I)	Perylo(1,2-b)oxirene-7,11-dione, 7a,8a,8b,8c,9,10-hexahydro-1,6,8c,9-tetrahydroxy-, (7aR-(7αalpha,8αalpha,8bbeta,8calpha,9αlpha))-	C ₂₀ H ₁₄ O ₇	366.324	102694-30-4
	Stemphytoxin III (STE III)	(7aR,8aR,8bS,8cR)-7a,8a,8b,8c-Tetrahydro-1,6,8c-trihydroxyperyleno[1,2-b]oxirene-7,11-dione	C ₂₀ H ₁₂ O ₆	348.309	102694-32-6

TABLE 1: Continued.

Group and chemical class	Mycotoxin	Chemical name	Molecular formula	Molecular weight (g/mol)	CAS number
Anthraquinones	Macrosporin A	1,7-Dihydroxy-3-methoxy-6-methyl-9,10-anthraquinone	C ₁₆ H ₁₂ O ₅	28,426.348	22225-67-8
	Altersolanol A (As-A)	(1R)-1,2,3,4-Tetrahydro-1,2 α ,3 α ,4 β ,5-pentahydroxy-7-methoxy-2-methyl-9,10-anthracenedione	C ₁₁ H ₁₆ O ₈	336.293	22268-16-2
Dihydroisocoumarin	Monocerin	(2S,3aR,9bR)-6-Hydroxy-7,8-dimethoxy-2-propyl-2,3,3a,9b-tetrahydro-5H-furo[3,2-c]isochromen-5-one	C ₁₆ H ₂₀ O ₆	3,083.264	30270-60-1
	Altenuic acids I, II, III	—	C ₁₅ H ₁₄ O ₈	—	—

Especially when combined with DON, TeA was found to significantly reduce the cytotoxicity of this mycotoxin [32]. TeA epimerizes to a mixture of TeA and allo-TeA when treated with bases and under acidic conditions. The presence of allo-TeA in fungal culture extracts (*Alternaria brassicicola*, *Alternaria raphani*, and *Phoma sorghina*) has been shown. TeA and allo-TeA phytotoxic effects can be explained by the inhibition of photosynthesis by blocking the electron flow, although there are no further toxicological data regarding allo-TeA in the published literature [31]. Different tautomers of 3-acetyl tetramic acid have been reported but it was not possible to distinguish between them. The TeA structure is still not clear, especially in an aqueous solution. Varying TeA structures are still widely used, resulting in different CAS registry numbers [33].

The perylenquinone derivatives, such as ATX I, ATX II, ATX III, Alterperyleneol (ALTCH; synonym Alteichin), and stemphytoxins (STE) are minor metabolites of *Alternaria* spp. but are considered to be very critical because of their mutagenic properties [34]. Because of the lack of available reference compounds, in particular for ATXs, analytical studies remain less common [11]. ATXs have been reported to be highly potent mutagens and more acutely toxic to mice than AOH and AME and cause DNA strand breaks. Recently, high genotoxic potency of ATX II in both mammalian and human cells was demonstrated, and it was described as the most potent substance within the ATX group, capable of different mechanisms of action [34]. Nevertheless, data concerning the underlying modes of action are still limited [20].

Alternaria alternata lycopersici toxins (AALs) exhibit mostly phytotoxic effects but have been shown to disturb the sphingolipid metabolism in a similar way to fumonisins, which have been correlated with esophageal cancer and other animal diseases [35, 36].

Altenusin (ALN) is a biphenyl derivative isolated from different species of fungi, which presents antioxidant properties and the ability to inhibit several enzymes, such as myosin light chain kinase, sphingomyelinase, acetylcholinesterase, HIV-1 integrase and trypanothione reductase, cFMS kinase, and pp60c-SRc kinase in the low micromolar concentration range, and it may serve as a chemotherapeutic agent to treat trypanosomiasis and leishmaniasis [37, 38]. The biphenyl basic skeleton of ALN containing a salicylic moiety and a catechol moiety could be the important part because of its interesting azole-synergistic activity.

Altersetin (ALS) was reported to possess broad antimicrobial activity against several multidrug-resistant bacterial demonstrating potent activity against several pathogenic Gram-positive bacteria and moderate *in vivo* efficacy in a murine sepsis model [39].

Anthraquinones and tetrahydroanthraquinone analytes are secondary metabolites widely distributed in natural biosources, which show significant biological activity. So far, several compounds of the alterporriol and altersolanol families have been reported, including the emerging *Alternaria* toxins macrosporin A and altersolanol A (As-A) [40], which exhibited antibacterial activity [41].

The cyclic tetrapeptide TEN is one of the major *Alternaria* toxins produced, along with dihydrotentoxin (DH-TEN) and

isotentoxin (iso-TEN). Their structures differ at the unsaturated bond of the N-methyldehydrophenylalanine moiety, which is hydrogenated into a single bond in DH-TEN and E configured in iso-TEN. All three compounds are considered to be phytotoxins, with TEN being the most potent, inhibiting photophosphorylation and inducing chlorosis. However, no toxicological data are available for mammals, and the data on the occurrence of this toxin in food and feed are limited as well [11].

The altenuic acids consist of three closely related isomeric colorless substances (altenuic acids I, II, and III) containing one carbon-methyl and one methoxyl group. The structures of the altenuic acids I and III remain unknown, but their molecular formula ($C_{15}H_{14}O_8$), which is identical to that of altenuic acid II, has been determined [42]. Williams and Thomas determined the chemical structure of altenuic acid II in 1973 by X-ray crystallographic analysis [43].

Monocerin is a polyketide metabolite isolated from several fungal species showing antifungal, phytotoxic, insecticidal, and plant pathogenic properties [44]. Monocerin and its analogues were proven to be nonspecific toxins and nonspecific seed germination inhibitors by their interference with selected stages of cell division cycles. In recent years, they have attracted greater interest; consequently, several syntheses of this molecule have been reported [45].

Other *Alternaria* metabolites were reported to be phytotoxins, that is, being toxic to plants, while the role of many others, such as infectopyrones, phomapyrones, and novaezelandins, is still unknown. However, *Alternaria* spp. produce a variety of other metabolites for which no reports are available due to the lack of pure substances, of which only AME, TeA, AOH, TEN, and ALT are now available on the market [12]. Figure 1 shows the chemical structure of the main *Alternaria* mycotoxins.

1.3. Stability of *Alternaria* Mycotoxins. The stability of *Alternaria* toxins has not been studied in detail. Current information reveals that *Alternaria* mycotoxins were barely degraded during wet baking, while significant degradation occurs upon dry baking, with stability decreasing in the ratio of AME > AOH > ALT [46]. Dibenzo- α -pyrones AOH and AME were stable to heating at 100°C in sunflower flour, while heat treatment at 121°C for 60 min significantly reduced the concentrations of these toxins in *Alternaria* contaminated sunflower flour. However, the heat-treated material caused some toxic effects when fed to rats [2]. AOH and AME were very stable in spiked apple juice at room temperature for up to five weeks and at 80°C after 20 min. They were also stable in spiked white wine for almost 8 days at room temperature. ATX-1 contents were stable when added to apple juice for up to 27 days at room temperature [10]. Overall, some *Alternaria* toxins remain difficult to degrade or decontaminate in fruits and their processed products.

1.4. Metabolism of *Alternaria* Mycotoxins. Mycotoxins can be partially metabolized in living organisms, leading to the formation of conjugated toxins by the conjugation of the parent compound with glucose, sulfates, and other sugar moieties. The term “masked mycotoxins” firstly appeared

FIGURE 1: Chemical structure of the main *Alternaria* mycotoxins.

defining a mycotoxin derivative that may be cleaved during digestion in living organisms to release its parent form. However, these conjugated mycotoxins are currently known as “modified mycotoxins” after a more recent comprehensive classification [47]. It is not clear if these modified mycotoxins can be hydrolyzed and absorbed in the gastrointestinal tract, thereby further contributing to the overall exposure. Since current information on the bioavailability of modified mycotoxins is very limited, EFSA has recommended to national agencies that they gather occurrence data on these modified forms using properly validated, robust, and sensitive routine analytical methods [48], a prerequisite being the availability of reference standards for these compounds. Over the last two decades, formation of glycosides and sulfated mycotoxins has been reported and (bio)organic synthesis has already been applied to obtain reasonable amounts of selected conjugates as reference materials for further studies [49]. Little information about the metabolism of AOH is available, but since all products of aromatic hydroxylation of AOH are catechols or hydroquinones, which can create reactive semiquinones and quinones or undergo redox cycle, its inactivation by methylation or glucuronidation/sulfation is considered to be a possible toxicological product that may cause toxic effects on cells lines [50]. In the mammalian organism, formation of glucuronides is a major pathway of detoxification and excretion. Both AOH and AME have free hydroxyl groups available for metabolic conjugation. Consequently, modified *Alternaria* mycotoxins such as alternariol-3-glucoside (AOH3G), alternariol-3-sulfate (AOH3S), alternariol monomethyl ether-3-glucoside (AME3G), and alternariol monomethyl ether-3-sulfate (AME3S) have received more attention during the last decade [49]. *In vitro* studies have shown that AOH and AME were readily converted to glucuronides upon incubation with hepatic and intestinal microsomes from humans, rats, or pigs in the presence of UDP-glucuronosyltransferases. AME was predominantly converted to the 3-O-glucuronide whereas AOH gave rise to comparable amounts of 3-O-glucuronide and 9-O-glucuronide [51]. The hydroxylation of AOH and AME was studied under *in vivo*-like conditions in precision-cut rat liver slices of rats. The pattern of *in vivo* metabolites was comparable to that of *in vitro* metabolites of AOH, clearly supporting the relevance of an oxidative *in vivo* metabolism [20]. AOH and AME were extensively conjugated in suspension cultures of tobacco BY-2 cells, demonstrating that masked mycotoxins of AOH and AME can be formed in plant cells [51]. Five AOH conjugates were isolated and identified by MS and NMR spectroscopy as β -D-glucosides (attached in AOH 3- or 9-position). For AME, conjugation resulted in β -D-glucoside (mainly attached in the AME 3-position) [52]. Knowledge of the toxicity and disposition of the oxidative AME and AOH metabolites is mandatory for a better understanding of the health risks posed by these *Alternaria* toxins, since toxicity properties may be enhanced or attenuated for their metabolites [53].

Toxicokinetic studies have so far focused on toxins with a dibenzo- α -pyrone structure, in particular, AOH and AME. Only very little information is available on the occurrence and toxicology of *Alternaria* toxins with a perylene

quinone structure, such as ATXs, ALTCH, and STEs. After studying the absorption of four *Alternaria* toxins with perylenequinone structures in the Caco-2 cell Transwell system (a widely accepted *in vitro* model for human intestinal absorption and metabolism), it was shown that ATX I and ALTCH were not metabolized in Caco-2 cells, while ATX II and STE III were partly biotransformed by reductive deepoxidation to ATX I and ALTCH metabolites, respectively [54]. Very low ATX-II absorption and partial metabolism of ATX-I was observed in the intestinal Caco-2 Transwell system [20].

A comparative toxicokinetic study showed that TeA was completely bioavailable after oral administration in both pig and broiler chicken. Absorption was deemed to be slower in broiler chickens ($t_{\max}$ 0.32 h in pigs versus 2.60 h in chickens) and TeA was more slowly eliminated in broiler chickens ($t_{1/2\text{el}}$ 0.55 h in pigs versus 2.45 h after oral administration). These observations were mainly due to the significantly lower total body clearance (Cl 446.1 ml/h/kg in pigs versus 59.2 ml/h/kg in chickens after oral administration) [55]. Concerning human exposure, the presence of TeA in the urine of six human volunteers in concentrations ranging from 1.3 to 17.3 $\mu\text{g/L}$ was reported. It was observed that 87–93% of orally consumed TeA was excreted as parent compound into the urine of two human volunteers within 24 h after ingestion [28].

2. Material and Methods

A systematic literature search was conducted using the databases Medline, Web of Science, and Scopus with the focus on the following keywords: *Alternaria* mycotoxins analysis, determination, occurrence, toxicity, stability, metabolism, etc. The period of time framed was 2005–2017. Fifty-eight articles, which met the criteria to be included into the study, were analyzed and classified. To facilitate data presentation three groups were established based on the analyzed food/feed matrices, namely, (i) cereals and cereal by-products, (ii) vegetables, fruits, and derived products (including juices, wine, vegetable seeds, spices, herbal infusions, and dry fruits), and (iii) mixed matrices studies, which combine the analysis of different foods belonging to both groups (i) and (ii), and products nonclassifiable into groups (i) and (ii). The information was double-checked to select bibliographies of relevant literature, and a thorough evaluation was performed to summarize the information about extraction method, analytical methodology, and studied mycotoxins limits of detection quantitation.

3. Results and Discussion

3.1. Analytical Methods for *Alternaria* Mycotoxins Determination. Mycotoxin regulations are based on risk assessment (hazard and exposure), the parameters of which are still hard to establish, meaning that a concrete interpretation of the consequences for consumer's health remains elusive. Furthermore, it remains difficult to detect toxin metabolites at low levels in complex food matrices. Therefore, validated analytical methods ensuring robustness, sensitivity, and reliability are needed.

In the last years, *Alternaria* toxins were usually extracted from solid or liquid matrices by the classic solid-liquid/liquid-liquid extraction (SLE/LLE) with organic solvents mainly acetonitrile (ACN; 68%), ethyl acetate (EtOAc; 17%), methanol (CH₃OH; 6%), and solvent mixtures such as ACN/EtOAc (2%). The chlorinated solvents dichloromethane (CH₂Cl₂; 2%) and chloroform (CHCl₃; 5%) were also used for SLE/LLE procedure. In the case of cereals and cereal by-products the choice of ACN as extraction solvent raised to 83%, followed by EtOAc (13%) and CH₂Cl₂ (4%), whereas in fruits and vegetables ACN represented 50%, followed by EtOAc (24%), CHCl₃ (12%), and CH₃OH (10%). For other food matrices not included in the mentioned groups the solvents used for extraction procedure were limited to ACN (75%) and CH₃OH (25%).

Although basic SLE/LLE represented approximately 50% of the extraction techniques for *Alternaria* mycotoxins analysis in food and feed, other used methodologies such as QuEChERS (14%), dilution and/or direct injection (11%), and the combination of SLE-SPE (14%) were of relevant importance. Direct SPE, microscale extraction, dispersive liquid-liquid microextraction (DLLME), and countercurrent chromatography (CCC) technique were also used (all <5%). Variations in the predominance of each methodology were observed depending on the analyzed food group. Thus, *Alternaria* mycotoxins were mainly extracted from cereals and derived products by SLE (64%) QuEChERS procedure (18%) and SLE-SPE (14%), while fruits and vegetables were mainly represented by SLE/LLE (43%), dilution-injection (16%), and SLE-SPE and direct SPE (both 14%). Other matrices or food combinations were mainly extracted by SLE/LLE and dilution-injection (both 29%), followed by direct SPE and SLE-SPE (both 14%).

With regard to mycotoxins determination, liquid chromatography (LC) was by far the most used technique, from other generally reported chromatographic techniques for, such as thin-layer chromatography (TLC) or gas chromatography (GC). High- and ultraperformance LC (HPLC and UPLC) have provided new possibilities allowing high-throughput screening by shortening the analysis time, while maintaining the chromatographic principles and improving the speed, sensitivity, and resolution [56]. In the early years, atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), and LC-tandem mass spectrometry (MS/MS) have become the methods of choice for the identification and quantification of these toxins [57].

Consequently, UPLC and HPLC, coupled to MS/MS (including high resolution MS; HRMS and time-of-flight MS; QTOF-MS), represented the 80% of the determinations, rising to 95% when only considering cereals and cereal by-products. Other detectors such as ultraviolet (UV; 7%) and diode array detection (DAD; 7%) were also used, followed by the enzyme immunoassay technique (EIA; 4%). In the case of *Alternaria* mycotoxins detection in fruits and vegetables the main representative one also was MS/MS (64%), followed by UV and DAD (both 14%) and finally EIA (7%).

Table 2, divided into cereals and cereal by-products (Table 2(a)), vegetables and fruits (Table 2(b)), and other food products (Table 2(c)), describes the analytical method,

extraction methodology, analyzed mycotoxins, and limits of detection/quantitation for the analyzed studies.

3.2. Prevalence of *Alternaria* Mycotoxins. In the last years different food matrices were analyzed for the presence of *Alternaria* mycotoxins, including cereals (wheat, barley, rice, oat, maize, sorghum, bakery products, and bread); fruits and vegetables (tomato, tomato products, pepper, garlic, onions, black radish, apple and apple juice, sweet cherry, strawberries, berries and blueberries, red fruits, orange fruits, citrus, and pomegranate fruits); beverages (beer; wine: red, white, and rosé; tea and herbal infusions; cider; fruit; and vegetable juices); other products (spices, nuts, walnuts, dried figs, olives, oilseeds, sugar beet pulp, maca, soy isoflavones, soya meal, and ginkgo biloba); silage, feed, and feed ingredients.

Among all of the analyzed matrices, tomato, followed by cereals and fruits, especially apples and berries, were the most studied foodstuff in recent years for *Alternaria* mycotoxins. *Alternaria* toxins are, together with aflatoxins, ochratoxin A, and patulin, the most commonly found mycotoxins in fruits and their processed products. Due to the limitations of current industrial processes to completely eliminate the rotten tissues and the reported stability of some *Alternaria* mycotoxins (i.e., AOH, AME, and ATXs) in fruit juices and during tomato processing, it is obvious that these mycotoxins are likely to be present in commercial end products [47].

Consequently, *Alternaria* mycotoxins were detected in cereals and derived products [46, 57–76], feed [74, 109–113], dairy products [114], grapes and by-products [27, 77, 115], tomatoes and derived products [31, 47, 77, 81, 106, 113], nuts and by-products [102, 116], spices [117], soybeans [76, 116, 118], sunflower seeds [85, 104, 107], vegetables [27, 101], vegetable oils [105], strawberries [3, 91, 93], red fruits [92, 101], several fruit juices [27, 72, 90, 99], peppers [119], apples [88, 90, 109], peanuts [109], tea and herbal infusions [86], wine [89, 103], beer [75], fermented beverages [96], and spices and food supplements [84, 87, 120, 121].

The maximum concentrations of *Alternaria* toxins reported in commercial food products ranged from 1 up to 8700 µg/kg. The highest levels were found in samples visibly infected with *Alternaria* rot, that is, in products obviously not suitable for human consumption. According to the European Food Safety Authority (EFSA), major contributors to *Alternaria* mycotoxins dietary exposure are grains and grain-based products, especially wheat. The highest levels of AOH and AME were found in lentils, oilseeds, and tomato paste, followed by fruit and vegetable juices, wines, cereals, and vegetables (tomatoes and carrots). The highest concentrations of TeA were reported for cereals, followed by commercial beers, while AOH, AME, TeA, and TEN were found in legumes, nuts, and oilseeds, particularly in sunflower seeds [6].

Moreover, it was observed that the cooccurrence of *Alternaria* toxins with other mycotoxins is plausible. Owing to the different physical and chemical properties of all the prevalent mycotoxins in fruits, the determination of trace amounts of all them in food represents an extremely challenging task [100].

TABLE 2
(a) *Alternaria* mycotoxins analysis in cereal and cereal derived products: analytical method, extraction method, analyzed mycotoxin, and limits of detection/quantitation

Matrix	Analytical method	Extraction method	Mycotoxins	LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)	Reference
Bread	HPLC-MS/MS	SLE: EtOAc	AOH	20	60	[46]
			AME	5	15	
			ALT	10	30	
Barley	U-HPLC-OrbitrapMS	QuEChERS	AOH ALT	— —	— —	[58]
Barley	LC-HRMS	QuEChERS	AOH AME	— —	— —	[59]
Wheat	HPLC-DAD	SLE: ACN/KCl, CH ₂ Cl ₂	AOH	—	10	[60]
			AME	—	10	
			ALT	—	50	
			TeA	—	60	
Wheat	LC-ESI-MS/MS	SLE: H ₂ O, EtOAc/HCl	AOH	0.75	2.5	[61]
			AME	0.1	0.3	
			TeA	2.5	7.5	
Wheat	UHPLC-MS/MS	SLE: ACN/H ₂ O	AOH	0.3	—	[62]
			AME	0.02	—	
			ATX-I	0.3	—	
			Infectopyron	n.d.	—	
			Macrosporin	0.5	—	
			TEN	0.15	—	
Wheat	HPLC-MS/MS	SLE: ACN/H ₂ O/FA	AOH	3	7	[63]
			AME	8	15	
			TEN	2.5	5	
Wheat	HPLC-MS/MS	SLE: ACN, SPE clean-up	AOH	1	4-5	[64]
			AME	5-6	15-20	
			TEN	0.5-0.7	2	
			TeA	0.1	0.3-0.5	
Wheat and wheat-based food	UPLC-MS/MS	SLE: ACN-SPE	TeA	2	—	[65]
			AOH	8	—	
			TEN	0.8	—	
			AME	0.2	—	
Maize	LC-MS/MS	SLE: ACN/H ₂ O/AA	AME	0.02	—	[66]
			Macrosporin	0.05	—	
			Monocerin	0.05	—	

(a) Continued.

Matrix	Analytical method	Extraction method	Mycotoxins	LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)	Reference
Maize	HPLC-MS/MS	SLE: ACN/H ₂ O/AA	AOH	—	—	[67]
			AME	—	—	
			TEN	—	—	
Maize and wheat	HPLC-ESI-MS/MS	SLE: ACN/H ₂ O	Macrosporin	—	—	[68]
			AOH	0.75	2.25	
			AME	0.1	0.3	
Rice	UPLC-ESI-MS/MS	SLE: ACN/H ₂ O	Macrosporin	0.05	0.15	[69]
			TEN	0.4	1.2	
			AOH	—	0.3	
Rice, oat flakes, and barley	UPLC-MS/MS	SLE: ACN/H ₂ O/AA, hexane	AME	—	0.3	[70]
			TEN	—	0.1	
			AOH	0.50–0.85	1.00–1.70	
			AME	0.67–1.32	1.35–2.52	
			ALT	1.19–2.21	2.38–4.43	
			TeA	0.61–1.18	1.21–2.35	
			TEN	0.46–0.91	0.93–1.82	
			ATX I	1.01–1.81	2.02–3.62	
			AOH3S	1.24–2.41	2.48–4.82	
			AOH3G	0.74–2.11	1.49–4.23	
			AME3S	0.79–4.16	1.58–8.32	
			AME3G	0.77–2.50	1.53–4.99	
Sorghum and millet	HPLC-ESI-MS/MS	SLE: ACN/H ₂ O/AA	AOH	—	—	[71]
			AME	—	—	
			TEN	—	—	
			ATX-I	—	—	
			TeA	—	—	
Sorghum and infant cereals	HPLC-MS/MS	SPE stable isotope dilution assay	Macrosporin A	—	—	[72]
			TeA	0.2	0.6	
			AOH	—	—	
Cereals	LC-DAD/ MALDI-TOF-MS	SLE: ACN/H ₂ O/AA-SPE	AME	—	—	[57]
			TEN	—	—	
			AOH	—	—	
Complex feed matrices	UPLC-MS/MS	QuEChERS	AOH	—	1.0–5.0	[73]
			AME	—	0.5–2.5	
			ALT	—	1.0–10.0	
			TEN	—	2.5–5.0	
Maize, wheat, feed, silage, and feed ingredients	U-HPLC-orbitrapMS	SLE: ACN/H ₂ O/AA	AOH	—	—	[74]
			AME	—	—	
			ALT	—	—	
			TeA	—	—	
			TEN	—	—	
Beer	HPLC-ESI-IT-MS	LLE: EtOAc	TeA	2	8	[75]

(a) Continued.

Matrix	Analytical method	Extraction method	Mycotoxins	LOD (μg/kg)	LOQ (μg/kg)	Reference
Soya beans	HPLC-UV	Modified QuEChERS, clean-up	AOH AME	8 16	24 48	[76]
(b) <i>Alternaria</i> mycotoxins analysis in vegetables and fruits: analytical method, extraction method, analyzed mycotoxin, and limits of detection/quantitation						
Matrix	Analytical method	Extraction method	Mycotoxins	LOD (μg/kg)	LOQ (μg/kg)	Reference
Tomato	LC-UV	SLE: CHCL3	AOH	1	5	[77]
			AME	0.6	2	
			TeA	2.6	11	
Tomato	LC-MS/MS	SLE: MeOH, derivatization, SPE Strata-XL	ALT	10–20	20–50	[78]
			AOH	2	5	
			TEN	1	2.5–5	
			TeA	10	20	
			AME	0.3–1	1–2	
Tomato	HPLC-DAD	SLE: acidified ACN, clean-up	AOH	3.94	—	[79]
			AME	0.074	—	
Tomato products	HPLC-MS/MS	QuEChERS	ALT	0.34	—	[31]
			TeA	0.38	1.28	
Tomato and tomato products	UPLC-MS/MS	SLE: acidified ACN, clean-up	allo-TeA	0.29	0.97	[80]
			AOH	4	—	
			AME	1	—	
			ALT	2	—	
			TEN	2	—	
			TeA	2	—	
			ATX-I	2	—	
Tomato and tomato products	LC-ESI-MS/MS	DLLME: ACN/CHCl3	AOH	1.4	3.5	[81]
			AME	1.4	3.5	
			TEN	0.7	1.75	
Tomato and pepper products	HPLC-MS/MS stable isotope dilution assay	QuEChERS	TeA	0.86	2.89	[82]
Sweet pepper	UPLC-MS/MS	SLE: EtOAc, clean-up	AOH	3.3	6.6	[83]
			AME	12	25	
			ALT	0.6	1.2	
Sweet pepper	LC-UV	SLE: CHCL3	AME	1.1	—	[84]
			AOH	2.9	—	
			TeA	2.8	—	
Sunflower seeds	LC-MS	SLE: MeOH	AOH and AME	1.25	—	[85]

(b) Continued.

Matrix	Analytical method	Extraction method	Mycotoxins	LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)	Reference
Tea and herbal infusions	UPLC-MS/MS	LLE: EtOAc/FA, NH ₂ -SPE, C18-SPE	AOH	5.8–13	12–26	[86]
			AME	20–30	41–60	
			ALT	1.3–2.1	2.6–4.1	
Maca, soy isoflavones, garlic, black radish, St. John's wort, and ginkgo biloba	UPLC-MS/MS	SLE: EtOAc, clean-up	AOH	8	25	[87]
			AME	30	100	
			ALT	2	6	
Apple	HPLC-DAD	Microscale extraction	AOH, AME, TEN	20	50	[88]
Apple juice and wine	HPLC-UV	CCC: EtOAc/H ₂ O	AME	0.04	—	[89]
			AME	0.03	—	
			TeA	0.14	—	
Apple juice concentrate	UPLC-MS/MS	Dilution-analysis	AOH, AME, ALT and TEN	—	1.0–5.0	[90]
Strawberries	HPLC-MS/MS	SLE: EtOAc	AOH	0.75	1.75	[3, 91]
			AME	2	3.5	
			TEN	0.25	0.75	
Blueberries	HPLC-UV	SLE: EtOAc/FA	AOH	6	10	[92]
			AME	2	4	
Berries and field samples	HPLC-MS/MS	SLE: ACN	ATX-I	—	—	[93]
			ALT	—	—	
			TeA	—	—	
Pomegranate fruits and juices	HPLC-DAD	QuEChERS	AOH	15	50	[94]
			AME	15	50	
			TEN	20	66	
Wine, fruit juices	HPLC-DAD	Direct injection or SPE, clean-up	AOH	2.0–6.0	3.3–10	[95]
			AME	0.1–2.0	2–3.1	
Wine (red, white, and rosé), cider (white and rosé), and their cork stoppers	LC-ESI-MS/MS	Dilution (ACN)-filtration	AOH	—	0.6–5.0	[96]
			AME	—	0.2–2.2	
Tomato products and apple	enzyme immunoassay	Dilution-centrifugation-analysis	AOH	1–2	—	[97]
Tomato products and apple	enzyme immunoassay	Dilution-centrifugation-analysis	TeA	25–150	—	[98]

(b) Continued.

Matrix	Analytical method	Extraction method	Mycotoxins	LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)	Reference
Tomato- and citrus-based foods	UPLC-ESI-MS/MS	SLE: ACN, SPE clean-up	TeA	1	4-5	[99]
			AOH	5-6	15-20	
			TEN	0.5-0.7	2	
			AME	0.1	0.3-0.5	
Tomato products, fruit, and vegetable juices	UPLC-MS/MS	QuEChERS	AOH			[47]
			AME			
			ALT			
			TeA			
			TEN	3.0-18.3	1.1-5.7	
			ATX I		9.8-61.5	
			AOH3S			
			AOH3G			
			AME3S			
			AME3G			
Tomato, apple, sweet cherry, and orange fruits	UPLC-MS/MS	SLE: ACN (NaCl)-SPE (MCX and NH2)	AOH	—	1	[100]
			AME	—	1	
			TeA	—	1	
			ALT	—	1	
Tomatoes, tomato products, bell peppers, onions, and soft red fruits	LC-TOF-MS	SLE: ACN/EtOAc	TEN	—	1	[101]
			AOH	7.4-17.4	14.8-34.8	
			AME	4.7-9.0	9.4-18.0	
			AME		3	
Nuts	UPLC-MS/MS	SLE: ACN/H2O/AA (79:20:1, v/v/v)	AOH	—	2.9	[102]
			AME	—	14	
			ATX-I	—	1.2	
			TEN	—		
Vegetable juices, fruit juices, and wine	HPLC-MS/MS	SPE	AOH	—	0.5-0.6	[103]
			AME	—	0.4-0.5	
			ALT/iso-ALT	—	2.2-3.1	
			TeA	—	0.9-1	
			TEN	—	0.9-1	
			ATX-I	—	0.8-1	
			ATX-II	—	1.2-1.3	
			AA-III	—	1-1.1	
			AALs	—	1.4-1.9	
			AS	—	0.8	

(c) *Alternaria* mycotoxins analysis in other food products: analytical method, extraction method, analyzed mycotoxin, and limits of detection/quantitation

Matrix	Analytical method	Extraction method	Mycotoxins	LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)	Reference
Fruit juices and spices and cereals	HPLC-MS/MS by stable isotope dilution assay	Derivatization, clean-up	TeA	0.15-17	0.5-50	[27]
Tomato, blueberry, walnut, and wheat	Semiquantitative UPLC-HRMS	Microscale extraction	AOH, AME, TEN, ATX, ALT, TeA, ALN, AIA, PyA	—	—	[12]

(c) Continued.

Matrix	Analytical method	Extraction method	Mycotoxins	LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)	Reference
Tomato, wheat, juice, and sunflower seeds	LC-MS/MS	SLE: MeOH, derivatization for TeA. SPE clean-up	AOH	—	10	[104]
			AME	—	1–10	
			ALT	—	10	
			TEN	—	1–5	
			TeA	—	5	
Tomato products, bakery products, sunflower seeds, fruit juices, and vegetable oils	HPLC-MS/MS	ACN/H ₂ O/FA. Dilute-and-shoot	AOH	0.2–2.8	0.6–9.3	[105]
			AME	0.04–0.4	0.1–1.2	
			TeA	3.6–34	12–110	
			ALT	0.8–24	2.5–81	
			isoALT	1.3–19	4.4–62	
			TEN	0.1–2.0	0.5–6.6	
			ATX-I	2.1–14	6.9–48	
			AAL TAI	2.8–5.4	9.3–18	
			AAL TA2	1.2–17	3.8–55	
			AOH	620–8080	2080–29920	
Beers, tomato products, apple juices, olive, and dried basil	LC/APCI-MS/MS	SPE	AME	160–1380	540–4600	[106]
			ALT	2220–12310	7400–41000	
			TEN	200–4140	680–13700	
			TeA	1220–7120	4060–23730	
			ALT	—	1.5–2	
Wine, apples, apple juices, tomatoes, tomato sauces, citrus, dried figs, olives, sunflower seeds, and cereals	UPLC Xevo-TQ-S MS	SLE: ACN-(MgSO ₄) SLE: ACN/H ₂ O/FA	AME	—	1–2	[107]
			AOH	—	2	
			TEN	—	2–2.5	
			TeA	—	5	
			TEN	0.18–0.99	0.54–2.94	
Bread, cereals, chips, juice, nuts, oil, sauce, seeds, and spice	LC-MS/MS	SLE: ACN/H ₂ O-(hexane)-SPE LLE: EtOAc-SPE	DH-TEN	0.19–0.35	0.62–1.05	[108]
			isoTEN	0.10–0.45	0.33–1.32	

3.3. Most Reported Mycotoxins. Among the 58 studies included in Table 2, more than 80% analyzed AOH and AME, and around 50% included TEN and TeA, followed by ALT (36%). These were followed by *Alternaria* toxins ATXs included in 16% of the analyzed studies, whereas only a few publications reported other compounds such as macrosporin (9%), monocerin, ALN, AALs, and infectopyrone (all <3%). Notably, limited information was found about pyrenochaetic acid A (PyA), alternarienonic acid (AIA), and alloTeA and isoALT, recently reported in food samples for the first time [31, 105] and metabolites such as AOH3S, AOH3G, AME3S, and AME3G [47, 69]. In this way, AOH, AME, TeA, and TEN have been shown to occur in food samples frequently [82, 97, 108], while the occurrence of ALT, isoALT, ATX-I, and AAL toxins is of much lower incidence, mainly due to shortcomings in current analytical methodologies.

Slight differences were observed based on the analyzed food matrices. Thus, AOH and AME (both 86%) were the most analyzed ones in cereals and cereal by-products, followed by TEN and TeA (59 and 41%, resp.). Other mycotoxins studied in these matrices were ALN (27%), macrosporin (23%), ALT (14%), and less extent monocerin and infectopyrone (both 5%). The same trend was observed in fruits, vegetables, and derived products, with predominance of AOH and AME (both 79%), followed by TEN (41%), TeA (38%), ALT (34%), and ATXs (14%).

4. Concluding Remarks

In the last years, several food matrices were analyzed for the presence of *Alternaria* mycotoxins, with tomatoes and derived products, cereals and cereals by-products, and fruits such as apples and berries as the most representatives. The most common extraction technique was SLE/LLE with several organic solvents mainly ACN, EtOAc, and CH₃OH. Other methodologies of relevant importance were QuEChERS, dilution-direct injection and SLE-SPE. LC-MS/MS systems were by far the most used analytical techniques for *Alternaria* mycotoxins determination. AOH and AME, followed by TEN, TeA, and ALT were the most analyzed mycotoxins in food and feed.

With regard to the widespread occurrence of *Alternaria* mycotoxins in various food and feedstuffs intended for human or animal consumption and their high toxicity, more toxicological studies are needed on the field, transport, storage, and processing stages. The monitoring of their incidence at low concentrations is warranted to find out the extent of human exposure to these contaminants, as well as the influence of cooccurring microorganisms and their produced metabolites on the subsequent enhancement or inhibition of *Alternaria* species growth. Considerable attention should be paid to the mycotoxin production process in order to take adequate measures for their suppression and decontamination within national and international programs. Moreover, it remains important to examine the potential correlation among these toxic compounds, as different toxicity profiles may occur. Furthermore, the action of purified molecules in various *in vitro* systems needs to be studied in depth. Fungal population genetics also deserve

attention in order to elucidate their role as mycotoxin producers and to understand the *Alternaria* secondary metabolism (producing parent or conjugated analytes) when subjected to different ecophysiological factors (mainly temperature and water activity), which are able to modulate their toxicological profiles. The use of predictive models to determine the factors that influence *Alternaria* mycotoxin contamination in the field and across the different processing stages may result in a notable decrease of this contamination throughout the production chain.

Although there are no specific international regulations for any of the *Alternaria* mycotoxins, EFSA has provided a scientific opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in food and feed [6]. Risk assessment related to food safety is frequently hampered by the lack of quantitative data. Up to now, results on *Alternaria* mycotoxins risk assessment proved to be inconclusive due to limited representative occurrence and toxicity data. However, application of the threshold of toxicological concern (TTC) approach indicated that there might be a possible risk for human health related to the presence of *Alternaria* toxins in foodstuffs. Maximum levels admitted for these toxins should be released by EFSA and regulated by the European Union in the near future. As these contaminants could be found in a wide range of food products, their current and future determination is essential for regulatory bodies with the purpose of improving the quality of products and preserving consumers' health.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This research was supported by the Ministry of Economy and Competitiveness of Spain (Grant no. AGL2013-43194-P). Laura Escrivá is grateful for the Ph.D. grant provided by the Ministry of Economy and Competitiveness of Spain (Grant no. BES-2014-068039).

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