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Alternaria in food products

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Alternaria is a fungal genus ubiquitous in the environment; many species are saprotrophs or plant pathogens, which can accumulate toxic metabolites in the edible parts of plants. Its species, as well as its mycotoxins have been isolated from a wide range of foods, such as cereals, fruits, vegetables, and their derived products. The aim of this work is to review its current taxonomy status, incidence of *Alternaria* species and mycotoxins in foods, control strategies and analytical methods, and to highlight the future needs for research in this field.

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Introduction

The genus *Alternaria* is, among the main mycotoxigenic fungi in foods, the one that has received less attention from research until the last decade; hence, estimating their public health impact has become rather difficult. However, due to its high prevalence in many food commodities, and of their toxins in food and food by-products, there has been a bloom of scientific research on this fungal genus in recent years.

Alternaria species are ubiquitous in the environment; many are saprotrophs or plant pathogens, affecting crops in the field, causing stem and leaf spot diseases, or spoiling the plant fruits or kernels in postharvest stage. As they are able to accumulate toxic metabolites in the edible parts of plants, their correct identification and classification is required to evaluate the risk associated with its presence in foods.

Alternaria has been isolated from a wide range of food products, such as small grain cereals, nuts, tomato fruits, olives, bell peppers, apples, berries, citrus fruits, among others, as well as their derived products.

Many obstacles remain to be overcome in order to achieve a full knowledge on this genus and its relevance in food products. Its taxonomy is, up to the present time, under discussion, without a general consensus in the scientific community. There are no official methods for detection of its mycotoxins in food products, as well as not enough data of their natural occurrence in staples and commodities. The toxicity of their broad range of secondary metabolites needs to be thoroughly investigated. All these items should be covered in the next years to be able to develop sensible legislation on susceptible foods and to establish prevention strategies to control the health risk associated with this genus.

The aim of this review is to provide an insight into the current status of: taxonomy, incidence of *Alternaria* spp. and its mycotoxins in foods, control strategies, modern methods of analyses, and to highlight the needs for further research in this field.

Taxonomy current status

The taxonomy of *Alternaria* has been discussed for many years and has undergone several revisions. The morphological diversity within *Alternaria* is considerable and great efforts were required to organize taxa into subgeneric species-groups and species. Before the incorporation of molecular techniques, the classification of its species was based on morphological characteristics under standardized growing conditions, regarding mainly colony and conidial aspects and conidial chain branching patterns. Based on these features, more than 270 species were described [1], many of which were of food origin. The species-group concept was defined, in order to simplify classification, as a group of taxa with similar patterns of sporulation and sharing a high degree of conidial morphological characters.

Another attempt of classification was based on the pathogens associated with a particular plant disease. Several *A. alternata* pathotypes have been described, and the term pathotypes or *formae specialis* has been used to describe species morphologically related to *A. alternata* infecting a particular host and synthesizing a host-specific toxin (HST), which would be responsible for fungal pathogenicity or virulence and diseases on host plants [2]. At least seven different *f. sp.* epithets can be found in the literature, of which most were raised to species level by Simmons in his manual [1,3].

In recent years, phylogenetic investigations have supported some of the main morphological groups described on Simmons' identification manual [1], on which relies the current classification of the genus [3,4,5••,6]. However, the small-spored *Alternaria* species with concatenated conidia, among which the main plant and postharvest pathogens are included, has not been supported by molecular studies up to now. Because of the minimal molecular variation existing between them, recent studies have proposed to comprise them in a single section, the *Alternaria* sect. *Alternaria* [3,5••]. This section consists of approximately 60 of the common small-spored species, *A. alternata*, *A. arborescens*, and *A. tenuissima*, among them. A comprehensive revision on *Alternaria* taxonomy has been recently published by Lawrence *et al.* [5••].

Polyphasic approaches, combining traditional morphology, molecular sequence analysis and secondary metabolite profiling, have been successful for the identification of large-spored, plant pathogenic *Alternaria* species. However, when carried out on small-spored, food associated species, have only achieved separation at species-group level [7••].

An accurate taxonomic framework is required by plant and human health organizations to identify and control the *Alternaria* species involved in disease and accumulating toxic metabolites in foods. The lack of consensus on this genus' taxonomy has generated confusions about the main species involved in crop diseases and food contamination, together with wrong associations between a mycotoxin and the producing species, or has even led to the common believe that *A. alternata* is the most widely spread small-spore species in foods. More efforts are needed in this direction to provide a solid taxonomic system that allows univocally identification of the small-spore species commonly distributed in foods, without sacrificing the information that would be lost by the reduction of a diverse group of species into a single section.

Alternaria secondary metabolites and related toxicity

Alternaria is a genus well known for its ability to produce a wide spectrum of secondary metabolites, including various phytotoxins related to plant pathogenesis, both host and non-host specific, and mycotoxins that can contaminate food products.

Among the many *Alternaria* secondary metabolites only a few are thought to pose a risk to human health. The tetramic acid derivative, tenuazonic acid (TeA), the dibenzopyrone derivatives, alternariol (AOH), and alternariol monomethyl ether (AME), and perylene derivatives altertoxins (ATXs) are considered the main *Alternaria* mycotoxins because of their known toxicity and their frequent presence as natural contaminants in food. Some studies also include altenuene (ALT) and tentoxin (TEN), although only cytotoxic activity has been proved for ALT, and TEN is a phytotoxin causing chlorosis in the seedlings of many plants [8].

Toxicological data available in the literature are limited to the above-mentioned metabolites; however, neither good bioavailability studies nor long term clinical studies have been performed on any of *Alternaria* mycotoxins. Data on whole animal studies are absent in the literature except for TeA. In relation to human health, AOH and AME have been associated with high levels of oesophageal cancer in China, and TeA with a haematological disorder in Africa. These compounds have all been reported as non-host-specific phytotoxins to several crops, together with other *Alternaria* metabolites; meanwhile, the role of many *Alternaria* compounds such as infectopyrones, phomapyrones and novae-zelandins, is still not fully understood.

Host-selective toxins (HSTs) produced by species of *Alternaria* are low-molecular-weight secondary metabolites with a diverse range of structures. Their phytotoxicity has been studied on susceptible cultivars, and some of them are chemically related. HSTs of the pathotypes of Japanese pear (AK-toxin), tangerine (ACT-toxin), and strawberry (AF-toxin) are structurally analogous metabolites and share an esterified decatrienoic acid (EDA) as their common structures [2,9].

However, food relevant *Alternaria* species are able to produce many more metabolites, for which there are no reports on function, toxicity, and it is not known if they can be produced in the plants. Moreover, new compounds synthesized by this genus are constantly being discovered from *in vitro f*ungal cultures in the search for new bioactive substances. The most recent reports include new perylenequinone derivatives from endophytic *Alternaria* sp. and *A. tenuissima* [10,11], a new AME isomer, the alternariol-10-methyl ether, together with capsaicin, from and endophytic *A. alternata* from *Capsicum annum* [12], two altenuene derivatives and one isocoumarin from *A. alternata* [13] and two new solanapyrones from endophytic *A. tenuissima* [14]. All these new compounds showed biological activity at certain levels; perylenequinones have shown toxic effects in plants, and mutagenicity in bacterial and mammal cells, with variable levels of bioactivity [10], AME isomer displayed a range of cytotoxicity against a panel of human cancer cell lines [12] the altenuene analogues and the solanapyrones showed weak to moderate antibacterial activity [13,14].

Alternaria incidence in foods

Many *Alternaria* species are commonly associated with several plant diseases, infecting the plant in the field during pre-harvest stages and reducing crop yield. Besides, this fungus has been found to be responsible for different diseases during the postharvest shelf-life of many different horticultural products. The distribution of *Alternaria* species in a wide range of agricultural products, such as cereals, fruits and vegetables is well known and it has been reported worldwide. However, their incidence in new food products is continuously added to the list of the previously known as susceptible to contamination with this genus. Table 1 shows the most recent reports (covering the years 2014–2016) of *Alternaria* species isolated from foods in different studies around the world.

Alternaria mycotoxins in food and food products

The contamination of crops with *Alternaria*, and the subsequent accumulation of their toxic metabolites in foods have been thoroughly discussed in the literature. However, more and more food products are being investigated for the presence of *Alternaria* mycotoxins, and the range of contaminated foodstuffs widens as knowledge advances on this field. More incidence data are needed, in order to determine which products are the main contributors to human exposure to *Alternaria* mycotoxins, and therefore, establishing limits for their presence in those particular foods. For, example, the risk posed by TeA in infant foods has been recently evaluated by Rychlik *et al.* [22].

In response to this need, several studies have been made in recent years, comprising a diverse spectrum of foods and foodstuffs. Not only new products have been investigated for the natural occurrence of *Alternaria* toxins, such as infant foods, beer, or several types of fruit juices, but new toxic compounds, whose toxicity is still not clearly elucidated, have been incorporated to these surveys. Besides the most common *Alternaria* mycotoxins, AOH, AME, and TeA, other compounds such as TEN, ALT, and ATXs are more frequently searched, and metabolites never investigated before, such as isoaltenuene, altenuisol, altenuic acid III, *etc.* have been reported for the first time in foods [23•,24•].

Moreover, the effect of different food processing methods on the fate of *Alternaria* mycotoxins in the final products has been recently investigated. Previous research studies on brewing have suggested that existent mycotoxin levels in the raw grain might increase in the malting process, as a result of promoted fungal growth, and mycotoxins may, to some extent, effectively overcome the brewing process and thus be transferred from malt into beer. However, no data was available on *Alternaria* mycotoxins. Recently,

Table 1

Recent reports of *Alternaria* species in foods (2014–2016).

Substratum (disease)[a]	Country	*Alternaria* species/species-group	References
Apple (core rot)	Greece	*A. tenuissima* *A. arborescens*	[15]
Blueberry	Argentina	*A. tenuissima* sp.-grp. *A. alternata* *A. tenuissima*	[7••] [16]
Cabbage	Italy	*A. alternata* sp.-grp. *A. arborescens*	[17]
Cauliflower	Italy	*A. alternata* sp.-grp.	[17]
Grape (bunch rot)	Italy	*A. alternata* *A. arborescens*	[18]
Pomegranate (fruit rot)	Greece and Cyprus	*A. alternata* *A. arborescens* *A. tenuissima*	[19]
Rocket	Italy	*A. alternata* sp.-grp. *A. arborescens* *A. japonica*	[17]
Tangerine and tangor (citrus brown spot)	China	*A. alternata*	[20]
Tomato (black mould)	Argentina	*A. alternata* sp.-grp. *A. arborescens* sp.-grp. *A. tenuissima* sp.-grp.	[7••,21]
Walnut	Argentina	*A. tenuissima* sp.-grp. *A. alternata* sp.-grp.	[7••]
Wheat	Argentina	*A. tenuissima* sp.-grp. *A. infectoria* sp.-grp.	[7••]

[a] When isolated from damaged food.

AOH was found in commercial beer samples from the German market, with 100% positive samples ($n = 44$). The range of contamination was low (0.23–1.6 μg/L, median 0.45 μg/L); nevertheless, the frequency of their occurrence justifies the survey of AOH in raw materials used for beer brewing [25].

Winemaking is also known to be non-effective in eliminating mycotoxins, but few data were available on the real incidence of *Alternaria* toxins in wines from different origin, which justified the recent investigations on several wine varieties all over the world [24•,26,27]. Similar results were observed with fruit juices, since pasteurization does not eliminate the toxins, and the process can even concentrate them, especially when mouldy fruit is incorporated [23•,24•,26].

Extrusion processing is used for producing a range of cereal products such as breakfast foods, snacks, and animal feed, many of which have shown contamination with *Alternaria* mycotoxins. The possibility of reduction of *Alternaria* toxins in wheat using the extrusion process was recently investigated by Janić Hajnal *et al.* [28]. The reduction level of mycotoxin concentration during extrusion processing is largely dependent on several factors, including the type of extruder, extruder temperature, screw speed, moisture content of the extrusion mixture and residence time in the extruder, as well as the type of mycotoxin and its initial concentration in the raw material. Optimal extrusion parameters for reduction of three *Alternaria* toxins were moisture content (w) = 24 g/100 g, feeding rate (q) = 25 kg/h, and screw speed (v) = 390 rpm, with a reduction of 65.6% for TeA, 87.9% for AOH and 94.5% for AME.

Table 2 summarizes the results from the main *Alternaria* mycotoxin surveys in food and foodstuffs during the 2014–2016 period.

Control strategies

Natural strategies are currently preferred for the control of fungal contamination, since the indiscriminate use of synthetic antifungals has led to the development of resistant strains, requiring higher doses of fungicides, with the consequent increase in toxic residues in food products. The genus *Alternaria* is not an exception; recently, Avenot and Michailides [35] found that *A. alternata* strains collected from commercial pistachio orchards had developed resistance to two fungicides applied in the field, cyprodinil and fludioxonil.

The work done about the influence of abiotic parameters on the growth of the fungus and mycotoxins biosynthesis offers the chance to use their combination to control them. Adequate temperature and humidity conditions during postharvest could prevent both growth and mycotoxin accumulation by *Alternaria* species in susceptible products [36–38].

Several plant extracts, and natural compounds have proved effective in reducing *Alternaria* spoilage of fruits and vegetables [8,39]. Edible composite coatings based on hydroxypropyl methylcellulose (HPMC), beeswax (BW), and sodium benzoate reduced the incidence and severity of *Alternaria* black spot on cherry tomatoes during cold storage [40]. Chlorogenic acid, a polyphenol found in tomatoes, inhibited the colonization by *A. alternata* by inhibiting AOH biosynthesis [41]. Yan *et al.* [42] could control *A. alternata* growth on cherry tomatoes by the application of rhamnolipids as an alternative to chemicals.

Induced plant resistance has also been studied as an alternative to classical pesticides. Citrus plants treated with hexanoic acid showed enhanced resistance against *A. alternata* [43]. In addition, biological control approaches were developed, alone or in combination with natural compounds. *Trichoderma* spp. have shown inhibitory activity against *A. alternata* [44]. The effect of rhamnolipids on the biocontrol of *A. alternata* by *Rhodotorula glutinis* was studied in the infection of cherry tomatoes [45]. The combination of *Cryptococcus laurentii* with BHT (benzothiadiazole), a systemic resistance inducer in several plants, was used to control strawberries postharvest black rot by *A. alternata* [46].

However, as most of these strategies have been studied *in vitro* or validated *in vivo* in laboratory scale, its efficacy in the field or in postharvest storage remains yet to be proved.

Novel methods of detection and quantification for *Alternaria* toxins

Based on the increasing need for incidence data, a bunch of new analytical methods have been recently developed for detection and quantification of *Alternaria* toxins in foods. New multitoxin methods are available and easier and more efficient clean-up procedures have been proposed for several food matrices.

Alternaria toxins are usually extracted from solid and liquid matrices with organic solvents or solvent mixtures. Extracts clean-up is performed by liquid–liquid partition or solid phase extraction (SPE). Some novelties in extraction and clean-up methodologies in recent years include QuEChERs extraction methods for the analysis of *Alternaria* toxins in pomegranates [47], and in fruit and vegetable juices and tomato products [33], as well as a pretreatment method with counter current chromatography (CCC) for enrichment and clean-up of trace *Alternaria* mycotoxins in wine and juice samples [26].

Detection and quantification are usually made by chromatographic methods. In previous years, HPLC with UV or fluorescence detection (FLD) were the most used techniques. Currently, they are increasingly being

Table 2

Natural occurrence of *Alternaria* mycotoxins in foods in the 2014–2016 period.

Food product	Country	Mycotoxin	Number of positive/total samples[a]	Range (μg/kg or μg/L)	Reference
Alcoholic beverages					
Beer	Germany	AOH	44/44	0.23–1.6	[25]
Wines	China[b]	AOH	11/12	0.04–0.70	[26]
		AME	11/12	0.03–0.08	
		TeA	1/12	0.31	
Red wine	The Netherlands	AOH	1/5	11	[27]
		TeA	3/5	<5.0–46	
	Germany	AOH	13/14	1.68–7.65	[24•]
		AME	13/14	0.80–1.45	
		TeA	14/14	1.63–44.4	
		TEN	10/14	1.01–1.47	
		AA-III	3/14	5.87–6.10	
		ATL	10/14	1.09–2.91	
White wine	Germany	AOH	4/11	0.65–1.19	[24•]
		TeA	8/11	1.87–60.0	
		AA-III	7/11	Traces	
		ATL	6/11	1.18–1.80	
Cereals and cereal based products					
Bakery products	Germany	AOH	8/9	Traces	[23•]
		AME	8/9	3.2	
		TeA	9/9	75–210	
		TEN	9/9	9.2–12	
Cereal grains	The Netherlands	AOH	1/14	5.2	[27]
		AME	1/14	3.0	
		TEN	14/14	2.0–14	
Oat flakes	Belgium	TeA	5/16	2.13–39	[29•]
Rice based cereal foodstuff	Belgium	TeA	22/31	1.90–113	[29•]
		TEN	11/31	3.6–15.6	
		AOH	6/31	1.83–2.97	
Wheat	Serbia	AOH	11/92	0.75–48.9	[30]
		AME	6/92	0.49–70.2	
		TeA	63/92	2.5–2676	
Durum wheat	Italy	AOH	23/74	8–121	[31]
		AME	19/74	9–48	
Dried products					
Dried figs	The Netherlands	TeA	5/5	25–2345	[27]
Dry chilli	Belgium	AME	2/35	69.72–222	[32]
Fruits and fruit juices					
ACE juice[c]	Germany	AOH	2/9	1.95–3.97	[24•]
		TeA	6/9	1.38–2.64	
		TEN	2/9	3.59–5.66	
Apples	The Netherlands	AOH	1/11	29	[27]
Apple juice	China	AOH	8/15	0.10–7.94	[26]
		AME	7/15	0.03–0.88	
		TeA	6/15	1.75–49.61	
	Germany	AOH	3/20	2.10–4.31	[24•]
		TeA	14/20	1.74–8.94	
		TEN	4/20	traces	
Apricot juice	Germany	AOH	2/2	4.20–4.86	[24•]
		AME	2/2	1.50–1.54	
		TeA	2/2	5.34–19.2	
		ATL	1/2	2.24	
Carrot juice	Germany	ALT	1/4	1.72	[24•]
Citrus juice	Germany	TeA	1/1	2.04	[24•]
		ALT	1/1	18.4	
		AA-III	1/1	2.71	
Currant juice	Germany	AOH	3/8	1.60–8.16	[24•]
		TeA	6/8	2.78–3.61	
		TEN	4/8	2.69–10.27	
		ALT	1/8	1.18	

Table 2 (*Continued*)

Food product	Country	Mycotoxin	Number of positive/total samples[a]	Range (μg/kg or μg/L)	Reference
Grape juice	Germany	AOH	7/8	1.58–6.45	[24•]
		TeA	6/8	1.94–2.70	
		TEN	8/8	2.50–8.07	
		ALT	1/8	3.95	
Grapefruit juice	Germany	AOH	2/4	0.81–1.06	[24•]
		AME	1/4	0.89	
		TeA	2/4	1.10–1.51	
Juices (fruits and vegetables)	Germany	ALT	1/23	traces	[23•]
		AOH	13/23	0.65–16	
		AME	10/23	0.14–4.9	
		TeA	12/23	21–250	
		TEN	11/23	1.0	
Multifruit juice	Germany	AOH	2/13	1.78–6.21	[24•]
		TeA	6/13	2.66–5.98	
		TEN	3/13	1.32–6.00	
		ATL	1/13	5.85	
Orange juice	Germany	AME	1/2	1.13	[24•]
		TeA	1/2	2.02	
Sour cherry juice	Germany	TeA	5/7	2.12–7.43	[24•]
Oilseeds and vegetable oils					
Olives	The Netherlands	TeA	1/10	5.3	[27]
Sunflower seeds	Germany	isoALT	1/11	Traces	[23•]
		ALT	1/11	Traces	
		AOH	6/11	16–39	
		AME	7/11	0.64–21	
		ATX-I	1/11	Traces	
		TeA	11/11	350–490	
		TEN	10/11	6.7–800	
	The Netherlands	TeA	5/5	85–449	[27]
		TEN	1/5	5.0	
Vegetable oils	Germany	AOH	9/19	6.0	[23•]
		AME	16/19	2.8–14	
		TeA	4/19	15	
		TEN	9/19	11	
Tomato products					
Tomato concentrate	Belgium	AOH	23/27	<3.5–31.0	[33]
		AME	18/27	<4.7–6.10	
		TeA	27/27	<3.3–174.3	
		TEN	10/27	<5.0–8.9	
		ALT	15/27	18.7–62.0	
Tomato juice	Belgium	AOH	20/28	<0.8–27	[33]
		AME	15/28	<0.9–3.3	
		TeA	28/28	3.7–333.1	
		TEN	18/28	<0.7	
		ALT	14/28	<1.6–6.1	
Tomato products	Germany	AOH	24/34	6.1–25	[23•]
		AME	27/34	1.2–7.4	
		TeA	31/34	52–460	
		TEN	9/34	Traces	
	Belgium and Spain	TeA	11/11	700–4800	[34]
Tomato sauce	Belgium	AOH	24/28	<1.4–41.6	[33]
		AME	22/28	<0.8–3.8	
		TeA	28/28	7.7–330.6	
		TEN	6/28	<1.8	
		ALT	9/28	<3.6–12.1	
	The Netherlands	AOH	4/8	<2.0–25	[27]
		AME	4/8	<1.0–7.8	
		TeA	8/8	66–462	

AOH, alternariol; AME, alternariol monomethyl ether; TeA, tenuazonic acid; ALT, altenuene; isoALT, isoaltenuene, TEN, tentoxin; ATX-I, altertoxin-I; AA-III, altenuic acid III; ATL, altenuisol.

[a] Positive samples include samples at traces or non-quantifiable levels.

[b] Samples include sorghum, rice, pine nuts, buckwheat, barley, medicinal, walnut, millet, and plum wine.

[c] Vitaminized fruit juice, mixture of orange and carrot juice.

replaced by HPLC–MS or HPLC–MS/MS. Even though quantification precision is sacrificed by MS detection, these methods have the advantage to allow simultaneous detection of up to more than 20 metabolites in the same sample. Given the need of incorporating more *Alternaria* toxins in the screening, these techniques seem a useful alternative for ambitious exploratory surveys.

In the 2014–2016 period, several LC–MS methods have been developed, such as a semiquantitative screening method for six mycotoxins (including AOH and AME) in different matrices (tomatoes, bell peppers, onions and soft red fruits) [34], and LC–MS/MS methods for detection of AOH, AME, and TeA in wheat [30], and in strawberries [48]. But the actual outburst during this period was the development of LC–MS/MS multitoxin methods, either for detection and quantification of *Alternaria* toxins alone or together with other fungal toxins commonly present in foods. As many as 12 *Alternaria* mycotoxins (AOH, AME, TeA, TEN, ALT, isoALT, ATX-I, ATX-II, ATL, AA-III, AAL TB_1, and AAL TB_2) can be detected and quantified by these methods in matrices as diverse as cereals, wine, fruits, juices, tomatoes, olives, oilseeds and oils [23•,24•,27,29•,31,33].

Conclusions

A considerable advance has been made in the recent years on the knowledge of the genus *Alternaria*, which had been disregarded in the past when compared with other toxigenic fungal genera. More research is necessary, especially in order to clarify its taxonomy in a unique, organized system; more toxicology studies are needed on its wide range of secondary metabolites, and more data on the incidence of its species and toxins in the different foods and food products are required.

All these information would be useful to establish safe limits for *Alternaria* toxic metabolites in foods in order to prevent health risk for human population and to develop efficient control strategies for this pathogen.

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