FOOD CHEMICAL CONTAMINANTS

Analysis of Agricultural Commodities and Foods for *Alternaria* Mycotoxins[1]

PETER M. SCOTT
Health Canada, Health Products and Food Branch, Food Research Division, Address Locator 2203D, Sir Frederick G. Banting Research Centre, Ottawa, ON, K1A 0L2, Canada

Fungi of the genus *Alternaria* are parasitic on plants and other organic materials. *A. alternata* is a frequently occurring species of particular interest because it produces a number of mycotoxins, including alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins I, II, and III (ATX-I, -II, and -III), and L-tenuazonic acid (TeA). Cleanup procedures of analytical methods for foods and foodstuffs include solvent partition, generally used for TeA, and solid-phase extraction columns for AOH, AME, and ATX-I. These *Alternaria* mycotoxins have been determined by TLC, GC, and more usually LC, mainly with ultraviolet detection, although fluorescence and electrochemical detection have also been used for *Alternaria* toxins other than TeA. A Zn^{2+} salt is usually added to the LC mobile phase for TeA. Recently, atmospheric pressure chemical ionization and electrospray LC/MS and LC–MS/MS have been applied to the determination and confirmation of AOH and AME in apple juice and other fruit beverages at sub ng/mL levels. Natural occurrences of AOH, AME, and in some cases other *Alternaria* toxins have been reported in various fruits, including tomatoes, olives, mandarins, melons, peppers, apples, and raspberries. They have been found also in processed fruit products such as apple juice, other fruit beverages and tomato products, wheat and other grains, sunflower seeds, oilseed rape meal, and pecans.

Alternaria fungi are commonly parasitic on plants and may cause spoilage of fruits and vegetables during transport and storage. *Alternaria alternata* produces a number of mycotoxins, including alternariol (3,7,9-trihydroxy-1-methyl-6*H*-dibenzo[*b,d*]pyran-6-one; AOH), alternariol monomethyl ether (3,7-dihydroxy-9-methoxy-1-methyl-6*H*-dibenzo[*b,d*]pyran-6-one; AME), altenuene (2α,3β,4aβ-tetrahydro-2,3,7-trihyd-roxy-9-methoxy-4a-methyl-6*H*-dibenzo[*b,d*]pyran-6-one; ALT), altertoxin I {[1*S*-(1α,12aβ,12bα)]1,2,11,12,12a,12b-hexahydro-1,4,9,1 a-tetrahydroxy-3,10-perylenedione}; ATX-I) and related perylene derivatives, and L-tenuazonic acid (5*S*,6*S*-3-acetyl-5-*sec*-butyl-4-hydroxypyrrolidone-2,4-dione; TeA). Chemical structures of these metabolites are shown in Figures 1–3. The first 2 of these metabolites were isolated in 1953 (1). Several reviews on the *Alternaria* toxins have been published (2–7).

When grown in culture, many isolates of *A. alternata* from various sources have been found to be toxic to laboratory animals (8). Of the mycotoxins isolated, ATX-I is the most acutely toxic in mice, AOH and AME are not very acutely toxic, and TeA has been shown to be acutely or sub-acutely toxic in several animal species. Of particular interest are studies indicating mutagenic and carcinogenic properties of culture extracts and metabolites of *A. alternata*. A culture of *A. alternata* on corn flour was found to be carcinogenic in rats, and culture extracts were mutagenic in various microbial and cell systems (9–11). It has been suggested that *A. alternata* might be one of the etiological factors for human esophageal cancer in Linxian, China (11). ATX-I, AOH, and AME are mutagenic, although there is evidence that mutagenicities of AME and ATX-I decreased on purification (4, 8, 10, 12–16). There are also reports of subcutaneous induction of squamous cell carcinoma in mice by human embryo esophageal tissue treated with AOH (12) and of subcutaneous tumorigenicity with NIH/3T3 cells transformed by AME (17).

Analytical methods for *Alternaria* toxins were last reviewed in 1984 by Schade and King (18). The present review will focus on the determination of the major mycotoxins of *A. alternata* and their natural occurrence in food and foodstuffs.

Extraction and Cleanup

The phenolic *Alternaria* toxins are usually extracted from solid foods with organic solvents such as dichloromethane (19), methanol, acetonitrile, or ethyl acetate (Table 1), while for TeA it is preferable to have an acidic extraction solvent (Table 2). Hexane (as a defatting solvent) and/or water may be incorporated into the extraction mixture. Cleanup may involve treatment with ammonium sulfate solution, lead acetate or sodium bicarbonate solution (a commonly used cleanup procedure for TeA), solvent partition, silica gel chromatography (Tables 1 and 2), or gel permeation chromatography (35). Solid-phase

Received May 10, 2001. Accepted by AP July 9, 2001.
[1]Based on a presentation given at the 114th AOAC INTERNATIONAL Annual Meeting, September 10–14, 2000, Philadelphia, PA.

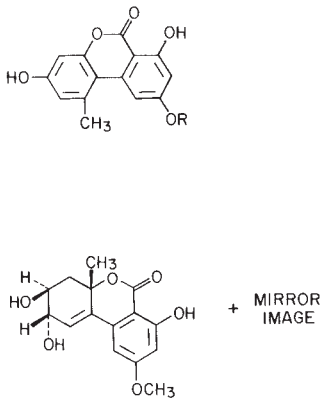

Figure 1. Structures of the phenolic *Alternaria* toxins AOH (R=H), AME (R=CH_3), and ALT (which is a mixture of optical isomers).

extraction (SPE) columns or cartridges have now found their place for extraction and cleanup of AOH and AME in liquid foods, such as apple juice, and of AOH, AME, ALT, and ATX-I in wheat extracts, but have not yet been used in methods for TeA (Tables 1 and 2). In our laboratory, C_{18} and aminopropyl SPE columns in series were used for cleanup of apple juice and other fruit beverages containing AOH and AME (27, 28) but poor recoveries of ATX-I were obtained from the silica-based C_{18} column. This problem could be overcome by using a polymer-based reversed-phase SPE column (29). A silica Sep-Pak column was, however, used for the wheat extracts without too much loss of ATX-I (recovery at an unspecified spiking level was 70%; 26).

Detection and Determination Techniques

The first method for analysis of *Alternaria* toxins in an agricultural commodity was for AOH and AME in harvested and stored tobacco, using thin-layer chromatography (TLC) for the initial screening and gas chromatography (GC) for determination (36).

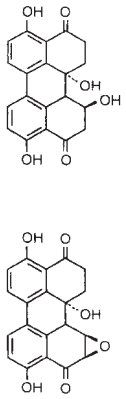

Figure 2. Structures of ATX-I (top) and altertoxin II (ATX-II; bottom).

Gas Chromatography

The use of GC for determination of *Alternaria* toxins has been reviewed (37). The GC of trimethylsilyl derivatives of AOH, AME, ALT, and TeA was first investigated by Pero et al. (38) and Harvan and Pero (39), but the lowest limit of quantitation for standards using flame detection was 100 ng. Detection by mass spectrometry (MS) has subsequently been proved to be much more sensitive (19, 40). TeA could be determined in tomato paste at a level as low as 6 ng/g by MS single ion monitoring (SIM) of its trimethylsilyl ether, and this technique showed separation of TeA from the isomeric D-*allo*TeA (31). A small amount of an analog of TeA, in which the 5-*sec*.butyl group is replaced by an *iso*propyl group, has also been observed in tomato products analyzed by capillary GC/MS (32). The capillary GC/MS of AOH, AME, ALT, ATX-I, and TeA trimethylsilyl and heptafluorobutyrate (HFB) full and partial derivatives were investigated in detail by Scott et al. (40). The most useful derivative of AOH was the *bis*-trimethylsilyl rather than the *tris*-trimethylsilyl derivative; SIM at *m/z* 402 gave a linear standard curve between 0.25 and 1 ng injected.

Thin-Layer Chromatography

TLC of *Alternaria* toxins has been reviewed briefly (18). Gradient high-performance TLC and densitometric determination of AOH and AME in raspberries, tomatoes, wheat, and oats was reported by Matysik and Giryn (20); however, the detection limit of the method was only 60 ng/g. Two-dimensional TLC on silica gel plates has proved more useful than one-dimensional TLC and has been used for determination of AOH, AME, ATX-I, and ATX-II in a number of agricultural commodities (34, 41–45); a quite low detection limit of 3 ng/g for AOH and AME in fruit and vegetable products was achieved using fluorescence in one study (41).

Liquid Chromatography

As GC and TLC have now been largely superceded by liquid chromatography (LC) for determination of *Alternaria* toxins in food extracts, the remainder of this review will concentrate mainly on LC as the determinative technique. *Alternaria* toxins can be detected after separation by LC, commonly reversed-phase LC, by UV, fluorescence, electrochemical detection, or MS (Tables 3–5). UV-visible absorption spectra at different pHs were published for AOH, AME, and ATX-I in a

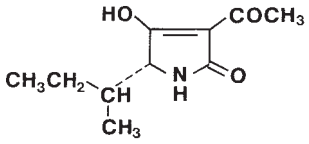

Figure 3. Structure of TeA.

Table 1. Recent extraction and cleanup procedures in methods for alternariol, alternariol monomethyl ether, and altertoxin I

Matrix	Extraction solvent	Cleanup	Reference
Cereals, tomatoes, and raspberries	Ethyl acetate	Aqu. $NaHCO_3$; silica gel chromatography	20
Tomato products	Methanol	10% Ammonium sulfate, then chloroform	21
Sunflower seeds and flour	Methanol	20% Ammonium sulfate, hexane wash; then chloroform partition	22, 23
Red pepper[a]	Methanol–hexane–HCl	Chloroform partition	24
Wheat[a]	Acetonitrile–4% KCl–HCl	Lead acetate; methylene chloride partition	25
Wheat[a]	Methanol	20% Ammonium sulfate, then methylene chloride; silica Sep-Pak	26
Apple juice and other beverages	—	SPE - C_{18}, aminopropyl	27, 28
Apple and grape juices[a]	—	SPE (Abselut)	29
Tomato paste	—	SPE (Oasis)	30

[a] Method includes altertoxin I.

study of their acid dissociation constants (68). A wavelength of about 256 nm at below pH 7 offers the greatest sensitivity in reversed-phase LC for AOH and AME; this has been used by several authors for UV detection of low nanogram and sub-nanogram amounts (Table 3). The technique leads to detection limits of the order of 1 ng/mL in an SPE method for AOH; AME in apple juice (27, 28). A typical chromatogram for reversed-phase LC of ATX-I, AOH, and AME standards at 256 nm in an acidic mobile phase is shown in Figure 4. When it is required to determine TeA, 280 nm is the wavelength of choice. A quantitation limit of 11 ng/g for TeA in tomato products was reported by da Motta and Soares (33). Diode array detection (DAD) of AOH, AME, ATX-I, ALT, and TeA was used to confirm these toxins in wheat extracts (25, 26), AOH, AME, ALT, *iso*ALT, ATX-I, and ATX-II in rice culture, and AOH in sunflower seeds (54), and to determine TeA in tomato juice (33). It is usual to add a metal ion chelating agent such as zinc sulfate to the mobile phase for TeA determination. Without this, TeA tails on reversed-phase LC because of binding to insufficiently demineralized column packing materials. However, Shephard et al. (58) were able to avoid this by using a deactivated end-capped octadecyl silica packing with a high carbon loading. The faster eluting *iso*propyl analog was detected in an extract of *A. alternata* culture. This is further evidence that this analog could be present in *Alternaria*-contaminated foodstuffs and it should be taken into consideration in analytical methods for TeA, in addition to D-*allo*TeA. Other workers have used ion pair (e.g., with tetrabutylammonium phosphate), anion exchange (e.g., with ethylenediaminetetraacetic acid), or ligand exchange chromatography (with 4-dodecyldiethylenediamine) for LC of TeA (Table 3).

Fluorescence has also been explored for determination of phenolic *Alternaria* toxins and for detection of ATX-I (Table 4), but is not applicable to TeA, which is not fluorescent. Emission responses for AOH, AME, and ALT at different excitation wavelengths in a reversed-phase solvent system are shown in Figure 5. Fluorescence is at least as sensitive as UV detection, but there can be interferences for AOH in apple juice extracts (24). A method for AOH in tomato paste had a low detection limit of 1.9 ng/g, but overall recoveries were not good for AME (30).

Electrochemical detection is also a very sensitive technique for LC of AOH, AME, and altertoxins (Table 5). The

Table 2. Typical extraction and cleanup procedures for tenuazonic acid

Matrix	Extraction solvent	Cleanup	Reference
Tomato paste	Methanol–hexane–10N H_2SO_4–methylene chloride	5% $NaHCO_3$, then acidification, back-extraction into methylene chloride	31
Tomato	Water–chloroform	Silica gel column; elute methanol cont. $ZnSO_4$	32
Tomato products	Methanol–hexane–water-c. HCl; chloroform	—	33
Olives	Methanol–water–hexane–c. HCl; chloroform	—	34
Wheat	Acetonitrile–4% KCl–HCl	Lead acetate; methylene chloride partitions; $NaHCO_3$ extraction	35
Wheat	Methanol, 20% ammonium sulfate, acidic methylene chloride	5% $NaHCO_3$ then acidification, back-extraction into methylene chloride	36

Table 3. Liquid chromatography of *Alternaria* toxins with UV detection

Toxin(s)[a]	Phase[b]	λ, nm	Min. det. amt., ng	Reference
AOH, AME	N	350	10	46
ATX-I	N	350	?	46
ATX-I	N	340	?	47
AOH, AME, ALT, TeA	N	280	60	48
AOH, AME	N, R	420	?	49
AOH, AME, ALT	R	254	?	50
AOH, AME	R	350	40, 20	51
AOH, AME	R	256	0.7, 0.5	27, 52
AOH, AME, ALT, ATX-I	R	276, 340	?	53
AOH, AME, ALT, ATX-I	R	240, 257, 340	3–10	54
AOH, AME	R	254	?	30
AOH, AME	R	254	?	28
AOH, AME, ALT, TeA	R	324, 278	?	55
AOH, AME, TeA	R (Zn^{2+})	280	?	32
AOH, AME, ALT, TeA	R (Zn^{2+})	257, 280	?	34
AOH, AME, ALT, TeA	R (Zn^{2+})	340, 280, 280	0.5, ?	56
AOH, AME, TeA	R (Zn^{2+})	254, 280	?	21
AOH, AME, ATX-I, ALT, TeA	R (Zn^{2+} for TeA)	257, 240, 280	?	26
ATX-1, TeA	R (Zn^{2+})	257, 280	?	25
AOH, AME, ATX-I, ALT, TeA	R (Zn^{2+} for AOH, AME, TeA)	257, 280	?	24
TeA	R (Zn^{2+}, C_{12}-DIEN)	280	2–5	31
TeA	R (Zn^{2+})	276	?	35
TeA	R (ion pair, anion exchange, ligand exchange)	280	?	57
TeA	R	277	0.4	58
TeA	R	284	2.6	33
TeA (2,4-dinitrophenyl-hydrazone)	R	330	?	59

[a] AOH, alternariol; AME, alternariol monomethyl ether; ALT, altenuene; ATX-I, altertoxin I; TeA, tenuazonic acid.
[b] N, normal phase; R, reversed phase.

topic has been reviewed by Visconti and Sibilia (5). AOH, AME, ATX-I, and ATX-II are electroactive and 0.01 to 0.05 ng could be detected by dual electrode coulometric ("screen mode") and single electrode amperometric detection techniques (62). In spite of this sensitivity, the techniques were only applied to detection and quantitation at sub-μg/g levels (in rice cultures, sunflower seeds, and mandarins) and the potential of the method for low ng/g quantitation has not been explored. ALT showed poor electroactivity. Visconti et al. (63) used dual in-series electrodes in the "redox mode" to improve sensitivity of the altertoxins relative to AOH; 35 ng ATX-I/g in tomato was readily measurable. In order to determine the altenuenes it was necessary to use post-column bromination (64).

Published reports on LC/MS of AOH and AME have appeared from a Chinese laboratory (65, 66), which used a particle beam interface and electron impact mode. However, the technique was insufficiently sensitive to detect nanogram amounts of these compounds. Our laboratory has investigated atmospheric pressure chemical ionization (APCI) and electrospray (ES) LC/MS and LC–MS/MS of AOH and AME as well as application of these techniques to their determination and confirmation in apple juice and other fruit beverages (67, 69). The MS detection techniques, used in conjunction with SPE cleanup (28), provide very sensitive methodology and determination of sub-ng/mL levels of AOH and AME in fruit beverages. An example is shown in Figure 6.

Other Techniques

Nonchromatographic procedures for determining *Alternaria* toxins are electrochemical-amperometric quantitation of AOH and AME with a carbon paste electrode incorporating mushroom tyrosinase (70) and electrooxidation of AOH, AME, and ATX-I (71). These techniques have not been applied to food analysis.

Table 4. Liquid chromatography of *Alternaria* toxins with fluorescence detection

Toxins[a]	Phase[b]	Excitation, nm	Emission, nm	Min. det. amt., ng	Reference
AOH, AME	N	?		1–2	46
ALT, ATX-1	N	?		?	46
AOH, AME	R	278	370, 389	0.05	32
AME	R	320	445	10	60
AOH, AME, ALT	R	315	430	?	61
AME	R	340	430	?	35
AOH, AME	R	330	430	0.05, 0.2	40
AOH, AME	R	330	430	1	30
AOH, AME	R	253	415	?	25
ALT	R	243	460	?	25

[a] AOH, alternariol; AME, alternariol monomethyl ether; ALT, altenuene; ATX-I, altertoxin I.
[b] N, normal phase; R, reversed phase.

Although no immunochemical methods have yet been developed for AOH and the other major mycotoxins of *A. alternata*, enzyme-linked immunosorbent assays (ELISAs) have been reported for the AAL toxins *(A. alternata* f. sp. *lycopersici* host-specific phytotoxins), which are structurally related to the fumonisins (72, 73). There are also LC methods for the determination of these phytotoxins in fungal cultures (74, 75). The *o*-carboxymethyl oxime derivative of TeA has been synthesized and conjugated to bovine serum albumin (76) but an immunoassay has not been developed for TeA.

Natural Occurrence of *Alternaria* Toxins

Natural occurrence of AOH, AME, TeA, and, in some cases, other *Alternaria* toxins (ALT, ATX-I) has been reported in various fruits and vegetables visibly infected by *Alternaria* rot, including tomatoes, olives, mandarins, melons, peppers, apples, and raspberries (Table 6). High levels of toxins were found in infected fruits and vegetables: apples, up to 58 800 ng AOH/g but only up to 500 ng TeA/g (77); tomatoes, up to 5300 ng AOH/g and 139 000 ng TeA/g (77); mandarins, up to 5200 ng AOH/g and 173 900 ng TeA/g (78); peppers, up to 440 000 ng AOH/g, 294 000 ng AME/g, 103 000 ng ALT/g, and 342 000 ng TeA/g (24); and olives, up to 2300, 2900, and 1400 ng/g AOH, AME, and ALT, respectively (34). These observations are useful in providing information on the relative occurrence of the toxins that might be found in processed fruit and vegetable products. Thus, ATX-I would not be expected to occur in tomato products to any significant extent. As a result of inoculation experiments, potential for the occurrence of *Alternaria* toxins in other fruits (oranges, lemons, and blueberries) has also been demonstrated (5).

The natural occurrence of *Alternaria* toxins in processed foods is of more interest from the human health viewpoint. TeA has been found occasionally in tomato products (Table 7), at levels up to 129 ng/g (21). However, the occurrence of AOH in processed fruit and vegetable products other than tomato products has been reported. In apple juice and other fruit beverages (Table 7), levels of AOH ranged up to 6 ng/mL (52, 67, 69). AME has also been detected in apple juice (traces) and in a sample of prune nectar. Further surveillance of fruit juices and other fruit and vegetable products for *Alternaria* toxins is needed to determine the level of human exposure from these foods.

Table 5. Liquid chromatography of *Alternaria* toxins with other detection systems

Toxins[a]	Detection	Detectable amts., ng	Reference
AOH, AME	Electrochemical	?	61
AOH, AME, ATX-I, ATX-II	Electrochemical	0.01–0.05	62
ATX-I, ATX-II	Electrochemical	0.03, 0.1	63
ALT	Br_2/electrochemical	8	64
AOH, AME	MS	1	65, 66
AOH, AME	MS, MS/MS	0.015	67

[a] AOH, alternariol; AME, alternariol monomethyl ether; ATX-I, altertoxin I; ATX-II, altertoxin-II; ALT, altenuene.

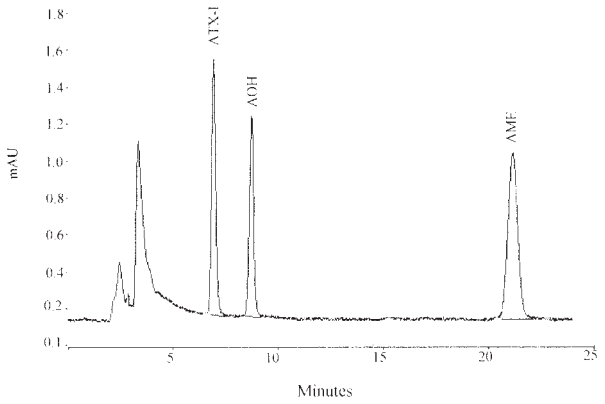

Figure 4. Liquid chromatogram of 5 ng ATX-I (6.9 min), 2.5 ng AOH (8.7 min), and 10 ng AME (21.2 min) detected by UV at 256 nm. Column: 250 × 4.6 mm Inertsil ODS-2; mobile phase: methanol–1% aqueous *ortho*-phosphoric acid (70 + 30, v/v); flow rate: 1 mL/min (S.R. Kanhere, unpublished results).

Alternaria toxins have also been found in several other agricultural commodities, including grains, sunflower seeds, oilseed rape, sorghum, and pecans (Table 8). Taking wheat as an important example, there are reports of AOH and AME in "black point" wheat in Poland at levels of up to 600 and 400 ng/g, respectively (85); AOH and AME in German wheat (up to 200 and 12 ng/g, respectively; 35); AOH, AME, and TeA in weather-damaged wheat in Australia at levels up to 224, 15, and 90 ng/g, respectively (26); AOH, AME, ATX-I, ALT, and TeA in Egyptian wheat (up to 2300, 1900, 1700, 1500, and 700 ng/g, respectively; 42); and of AOH, AME, and TeA in weathered wheat from China (maximum levels 335, 1426, and 6430 ng/g, respectively; 25). In the Chinese wheat, there were good linear regressions of correlations between concentrations of AOH and AME (r = 0.850) and between concentrations of [AOH + AME] and TeA (r = 0.796), indicating coproduction of the toxins in the field (25). Processing (milling) studies and methods for analysis of processed products such as flour would be useful. As another example, sunflower seeds have been shown to contain *Alternaria* toxins in Argentina (up to 792 ng AOH/g, 836 ng AME/g, and 31 600 ng TeA/g; 22, 83, 84) and in Italy (up to 1840 ng AOH/g and 129 ng AME/g; 78). Some studies on the effects of processing on AOH, AME, and TeA in sunflower products have been performed. Levels of AOH and TeA decreased during ensiling sunflower seeds (22); about half the AME, but no AOH and only 2% of the TeA, was transferred from sunflower seed meal into oil (23). On heating sunflower flour at 100°C for 90 min, AOH and AME were stable but half the TeA was lost (84). Because sunflower seeds are commonly eaten with

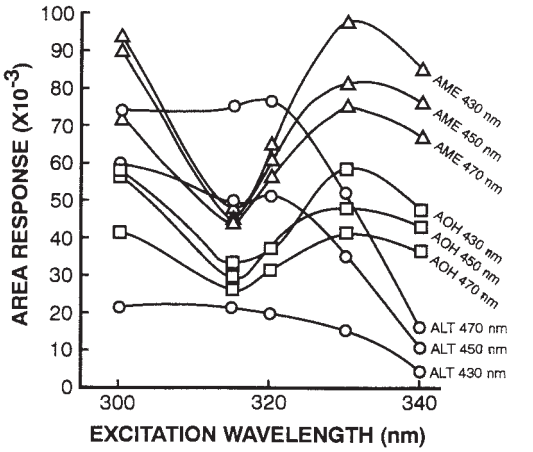

Figure 5. Fluorescence emission responses (peak area counts) of AOH (1 ng), AME (2.5 ng), and ALT (0.2 ng) at different excitation wavelengths in methanol–acetonitrile–1% aqueous *ortho*-phosphoric acid (1 + 1 + 1) using a Shimadzu RF-551 detector (S.R. Kanhere, unpublished results).

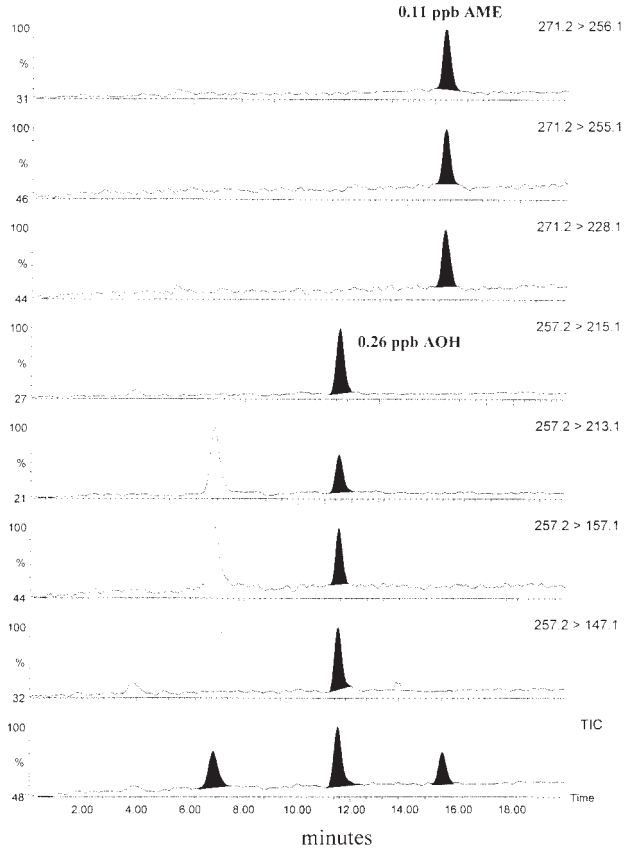

Figure 6. Reversed-phase LC–MS/MS of sub-ng/mL levels of AOH and AME naturally occurring in apple juice (B.P.-Y. Lau and D.A. Lewis, unpublished results). The peak at 6.9 min was not identified.

Table 6. Occurrence of *Alternaria* toxins in fruits and vegetables

Fruit	AOH	AME	ALT	ATX-I	TeA	Reference
Apple	+	+	(+)[a]	+	+	77
Apple	+	+	—[b]	—		53
Mandarin	+	+	—	—	+	78, 79
Melon	—	+	—	—	+	78
Olive	+	+	+	—	+	34, 78
Red pepper	+	+	+	—	+	24
Pepper	+	+	—	—	+	78
Tomato					+	80
Tomato	—	—	+			19
Tomato	+	+		—	+	78
Tomato	+	+		—	+	81
Tomato	(+)	(+)			+	32
Tomato	+	(+)	(+)	—	+	77
Tomato	+	+				41
Redcurrant	+	+				41
Raspberry	+	+				41
Strawberry	+	—				41
Gooseberry	+	—				41
Blackberry	+	—				41

[a] (+) = only trace levels and/or very low incidence.
[b] — = toxin looked for but not detected.

Table 8. Occurrence of *Alternaria* toxins in other foodstuffs

Foodstuff	AOH	AME	ALT	ATX-I	TeA	Reference
Pecan	+	+				82
Sunflower seed	+	+				62, 78
Sunflower seed	+	+				83
Sunflower seed	+	+			+	84
Wheat, triticale, oats, rye, barley	+	+				85
Wheat, barley,oats	+	+				35
Wheat	+	+	—[a]	—	+	26
Wheat	+	+	—	—	+	25
Wheat	+	+	+	+	+	42
Maize	—	—	+	—	+	42
Barley	—	+	+	—	—	42
Rice plants					+	86
Rice	—	—	+	—	+	42
Wheat bran	+	+	—	—	+	42
Sorghum	—	—	—	+	+	42
Sorghum	—	—			+	26
Sorghum	+	+	(+)[b]	(+)	—	87–89
Sorghum, ragi	—	+	+	—	+	43
Oilseed rape meal	+	+	—	—	+	90
Oilseed rape	—	—		—	—	44, 45

[a] — = toxin looked for but not detected.
[b] (+) = only trace levels and/or very low incidence.

Table 7. Occurrence of *Alternaria* toxins in processed foods

Food	AOH	AME	TeA	Reference
Tomato paste			+	31
Tomato products	(+)[a]	—[b]		41
Tomato products			+	21
Tomato paste			+	33
Raspberry drink	(+)	—		41
Apple juice	+	—		40, 69
Apple juice	+	(+)		67
Apple juice concentrates	+	(+)		52
Grape juice, cranberry nectar, raspberry juice, red wine	+	—		67, 69
Prune nectar	+	+		67, 69

[a] (+) = only trace levels and/or very low incidence.
[b] — = toxin looked for but not detected.

minimal processing, their analysis as packaged for the consumer is needed.

Conclusions

More work is needed on the development of reliable methods for the determination of *Alternaria* toxins in foodstuffs and foods. Immunochemical methods, both for screening purposes and for cleanup, are not yet available and would assist greatly in this effort. None of the methods outlined in this review have yet been subjected to interlaboratory study. However, they are sufficiently useful to have demonstrated considerable natural occurrence of these toxins in foodstuffs. The information on their occurrence in actual foods is limited so far to fruit products but indicates the possibility of a more widespread contamination of these foods. Foods made from other agricultural products such as wheat need to be analyzed. Effects of food processing on these toxins (and any transformations) have been little studied, and toxicology data are insufficient. Monitoring of foods using reliable methods is necessary in order to provide information on intake of these

toxins by consumers, and will give impetus to further toxicological studies if occurrence of *Alternaria* toxins in foods becomes a concern. In any event, their presence in foods might be used as an indicator of quality.

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