Occurrence of alternariol, alternariol monomethyl ether and tenuazonic acid in Argentinean tomato puree

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Abstract

The occurrence of *Alternaria* mycotoxins was investigated in 80 samples of tomato puree processed and sold in Argentina. Alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TA) were searched for by liquid chromatography. Thirty-nine of the 80 samples showed mycotoxin contamination. TA was found in 23 samples (39-4021 µg/kg), AOH in 5 samples (187-8756 µg/kg), and AME in 21 samples (84-1734 µg/kg). Co-occurrence of two of these toxins was detected in 10 samples. This is the first report of natural occurrence of AOH, AME and TA in tomato products in Argentina.

Keywords: tenuazonic acid, alternariol, alternariol monomethyl ether, tomato puree, *Alternaria*

Introduction

Tomato products are largely consumed in Argentina as tomatoes constitute one of the vegetables with the highest processing volume in our country and in the rest of the world (1). The area dedicated to this culture in Argentina is about 17,500 hectares (43,240 acres), with an average yield of 671,000 tons (2). The production is destined to fresh consume and to industrialized products (tomato puree, pulp, juice, sauces, etc.). Around 60% of the industrialized tomato fraction is used in the elaboration of tomato paste, which is reprocessed by the adding of water, spices and other ingredients, and then is packed and sold as "tomato puree".

The genus *Alternaria* contains numerous species that are both saprophytic and pathogenic on many plants, including several plants used for food (3). Because of their thin skin, tomatoes are very susceptible to fungal decay, and *Alternaria* is the most common fungus on moldy tomatoes (4). *A. alternata* is the causal agent of Blackmould of ripe tomato fruits, a disease frequently causing substantial losses of tomatoes, especially those used for canning (5). During the colonization of the host, the pathogen can produce toxic metabolites in the infected plants and when they accumulate in plant parts used as food, they can be a hazard to human health (3). Species of *Alternaria* are known to produce at least 70 secondary metabolites. The major *Alternaria* mycotoxins belong to three structural classes: the tetramic acid derivative, tenuazonic acid; the dibenzopyrone derivatives, alternariol, alternariol mono-methyl ether and altenuene; and the perylene derivatives, the altertoxins. Some species, in particular *A. alternata*, can produce mycotoxins in infected plants (6). High levels of toxins were found in fruits and vegetables (apples, mandarins, peppers and olives). *Alternaria* toxins have also been found in several other agricultural commodities, including wheat, sunflower seeds, oilseed rape, sorghum and pecans (7).

Sixteen of seventeen strains of *A. alternata* from black mould tomatoes collected in Italy produced *in vitro* high amounts of TA (up to 4200 mg kg $^{-1}$), AOH (600 mg kg $^{-1}$), AME (100 mg kg $^{-1}$), ALT (30 mg kg $^{-1}$) and ATX-I (13 mg kg $^{-1}$) (6). In Argentina, all the strains isolated from black mould tomatoes (eighteen *A. alternata* and six *A. tenuissima*) produced AOH and AME and sixteen of them produced TA *in vitro*. All the strains were able to retain

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their toxigenic capacity when they were inoculated in surface disinfected tomatoes (8). In naturally infected tomato fruits collected in southern Italy, *Alternaria* mycotoxins were present as TA (up to 7200 ng kg $^{-1}$), mainly associated with AME (up to 270 ng kg $^{-1}$) and AOH (up to 1300 ng kg $^{-1}$) (6).

AME and AOH are not very acutely toxic, but they have mutagenic effects. There is also some evidence of carcinogenic properties. Tenuazonic acid is not mutagenic in bacterial systems. However, it is toxic to several animal species; in dogs, it caused hemorrhages in several organs, and in chickens sub-acute toxicity was observed (9). Recently, liver and kidney damage in rats was produced by feeding *A. alternata* for 28 days (10). *A. alternata* was associated with human esophageal cancer in Linxian, China (11). Additional toxicological studies are clearly needed. There are no specific regulations for any of the *Alternaria* toxins in foods.

Considering that *Alternaria* spp. are the most frequent fungal species invading tomatoes (12) and its toxigenic capacity, the presence of their mycotoxins in tomato products should be evaluated in order to determine if a potential risk exists to the health of the consumer. The aim of this work is to focus the attention on the potential hazard of co-occurring toxic fungal metabolites in tomato products.

Materials and Methods

Samples

80 samples of tomato puree from 23 brands processed and sold in Argentina were analysed. The samples were acquired in local markets and supermarkets during the period of March-April 2006, in the city of Buenos Aires, Argentina. Most products contained as ingredients: tomato, salt and citric acid. The samples were kept at room temperature until analysis.

Determination of Alternaria toxins

The methods for the detection of *Alternaria* toxins in tomato products were described by da Motta and Valente Soares (13, 14). A 50 g portion of tomato puree was weighed and transferred to a blender cup with 150 ml methanol. It was blended at low speed for 3 minutes. An additional 50 ml methanol was used for washing the residues left in the blender cup into a beaker, and 60 ml of a 10% ammonium sulphate solution was added. The mixture was shaken and filtered to a separating funnel, then defatted with 40 ml hexane. Fifty ml of water at 8 ºC or below were added to the methanolic phase in order to avoid forming an emulsion. Two extractions with 40 ml chloroform were made. The chloroform extracts were collected, filtered through anhydrous sodium sulphate, and evaporated in a rotary evaporator at 35 ºC. The residue was dissolved in 4 ml HPLC grade methanol and was analysed for alternariol and alternariol monomethyl ether.

The methanolic phase was acidified to pH 2 with HCl (c). Two extractions with 40 ml chloroform were made. All the chloroform was collected in a separating funnel and washed with 30 ml water. The chloroform extracts were collected, filtered through anhydrous sodium sulphate, and evaporated in a rotary evaporator at 35 ºC. The residue was dissolved in 4 ml HPLC grade methanol and was analysed for tenuazonic acid.

Liquid chromatography

The HPLC system consisted of a Shimadzu LC-CA liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a Rheodyne sample valve fitted with a 20 µl loop and a Shimadzu UV detector Model SPD-6A. The analytical column was Jupiter 4.6 x 250 mm 5µ C18 (Phenomenex, USA). The mobile phase was methanol/water (80:20) containing 300 mg $ZnSO_4.H_2O$/l, for AOH and AME, and methanol/water (90:10) containing 300 mg $ZnSO_4.H_2O$/l, for TA. A flow rate of 0.4 ml/min was used. The wavelength for recording chromatograms was 258 nm for AME and AOH, and 280 nm for TA. A calibration curve was constructed for quantification purposes using the toxin standards and correlating peak-area versus concentration. The detection limits were 2.6 µg/kg, 0.6 µg/kg and 1.0 µg/kg for TA, AME and AOH respectively. The method quantification limits were 11 µg/kg for TA, 2 µg/kg for AME, and 5 µg/kg for AOH. The average recoveries for seven levels of addition of pure standards to tomato paste were 98.7% and 84.1% for AME and AOH, respectively. The average RSDs between duplicates for 14 sample preparations, for spiked tomato product samples were 0.8% for AME and 5.4% for AOH. The average recovery for six levels of addition for TA was 88%. The average RSDs between duplicates for spiked and naturally contaminated tomato product sample was 8.9% for 18 sample preparations for TA (13, 14).

The peak identity was confirmed by means of co-injection with the corresponding standard, and by using a Shimadzu SPD-M10Avp photodiode array detector. The spectra were acquired in the range of 200-300 nm. Reference spectra were acquired during the elution of associated standards and used for peak identification by comparison after spectra normalization.

Results and Discussion

Thirty-nine of the 80 samples (49%) were contaminated with *Alternaria* toxins (Table 1).

Table 1. Natural occurrence of tenuazonic acid (TA), alternariol (AOH) and alternariol monomethyl ether (AME) in tomato puree samples

Sample	TA (µg/kg)	AOH (µg/kg)	AME (µg/kg)
1	86	nd	nd
2	89	nd	nd
3	88	nd	nd
4	109	nd	nd
5	1428	nd	nd
6	1000	187	nd
7	430	nd	nd
8	1608	nd	782
9	136	nd	nd
10	nd	347	385
11	nd	nd	645
12	nd	nd	160
13	nd	nd	1477
14	2924	nd	nd
15	1653	nd	nd
16	nd	8756	nd
17	4021	nd	200
18	nd	nd	278
19	nd	nd	539
20	nd	1558	84
21	nd	nd	1332
22	nd	nd	702
23	nd	nd	804
24	nd	nd	138
25	123	nd	210
26	nd	nd	394
27	nd	nd	764
28	nd	nd	561
29	689	nd	191
30	660	nd	1734
31	1249	249	nd
32	746	nd	452
33	350	nd	nd
34	nd	nd	629
35	87	nd	nd
36	113	nd	nd
37	141	nd	nd
38	39	nd	nd
39	53	nd	nd

nd· not detected. <11 µg/kg for TA, <5 µg/kg for AOH, and <2 µg/kg for AME.
The results represent the average of two determinations

TA was found in 23 samples (29%) at levels ranging from 39 to 4021 µg/kg; AOH in 5 samples (6%) at levels from 187 to 8756 µg/kg, and AME in 21 samples (26%) ranging from 84 to 1734 µg/kg.
In this study, 10 samples were contaminated by two *Alternaria* toxins simultaneously. Six samples contained both TA and AME, 2 samples TA and AOH and 2 samples AME and AOH, respectively. In none of the 80 samples was determined the co-occurrence of the three toxins. Typical chromatograms are shown in Figures 1 and 2.

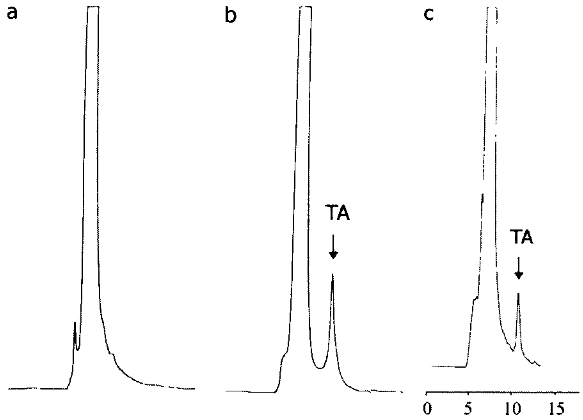

Figure 1. Chromatograms of (a) uncontaminated tomato puree sample; (b) tomato puree spiked with 500 µg/kg TA; (c) tomato puree sample naturally contaminated with 350 µg/kg TA

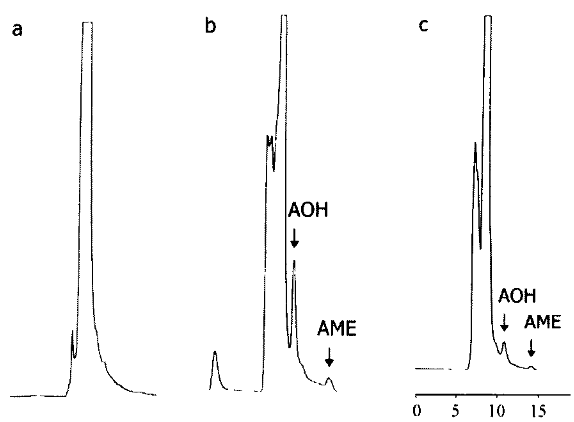

Figure 2. Chromatograms of (a) uncontaminated tomato puree sample; (b) tomato puree spiked with 1000 µg/kg AOH and 500 µg/kg AME; (c) tomato puree sample naturally contaminated with 347 µg/kg AOH and 385 µg/kg AME

The most frequent toxin was TA and it was found in significantly high levels. The range of TA found in the present work was lower than the results of Stinson *et al.* (15) (10.7-139 mg/kg) in whole tomatoes, and higher than those of da Motta and Valente Soares (16) (29-76 μg/kg) in tomato puree samples. There are few data on natural occurrence of AOH and AME in tomato and tomato products. Da Motta and Valente Soares (16) reported that no AOH or AME was detected in 80 samples of tomato products. Stinson *et al.* (15) found AOH and AME in whole tomatoes, although it was detected in few samples and in much smaller amounts than TA (AOH: 300-5300 μg/kg; AME: 100-800 μg/kg). These levels of contamination are similar to those found in this study.

The intake of tomato industrialised products in Argentina has been estimated by private sources in 10 kg per person per year. Tomato puree is the major product consumed (49%), followed by whole stewed tomato (35%), tomato sauces (13%) and tomato paste (2.7%) (1).

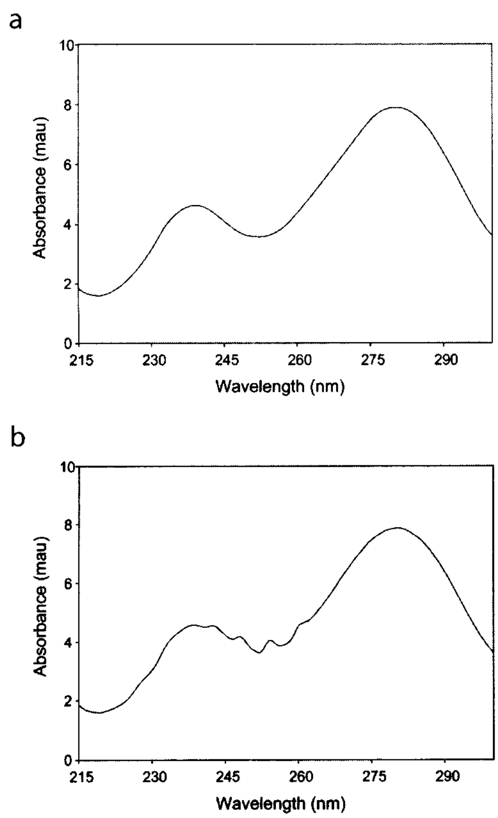

Figure 3. Spectra of tenuazonic acid (TA); (a) standard; (b) tomato puree sample naturally contaminated with 86 μg/kg TA, confirmed by photodiode array UV detector

Consumption of tomato products containing mycotoxins produced by *Alternaria* may represent a hazard to public health, particularly since these compounds have synergistic toxicity (17).

Conclusions

This is the first report of the natural occurrence of AOH, AME and TA in tomato puree processed and sold in Argentina. It is also the first time that AOH and AME have been reported as natural contaminants of tomato products in Latin America.

Alternaria spp. are the most frequent fungal species invading tomatoes (18, 19). In previous studies (8), a considerable amount of toxigenic *Alternaria* spp. strains has been isolated from whole fresh Argentinean tomatoes, indicating a high possibility of contamination of this product. Direct consumption of mouldy tomatoes by the consumer is unlikely, but the possibility of mouldy tomatoes being included in processed tomato products is much more likely. The results of the present work might indicate the use of fruits contaminated with their toxins for the elaboration of processed products.

Systematic testing of mycotoxins in tomato products is needed to increase their quality and safety. Furthermore, the presence of TA, AOH and AME in tomato products may be considered an indicator of the quality of raw material.

Acknowledgements

Universidad de Buenos Aires and Universidad de Quilmes supported this work.

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