Occurrence of *Alternaria* mycotoxins in food products in the Netherlands

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Background

The European Food Safety Authority (EFSA) concluded in their Scientific Opinion in 2012, in which they that *Alternaria* toxins are of high concern for public health. They can induce harmful effects in animals, including carcinogenic and teratogenic effects. *Alternaria* toxins can contaminate cereals, oilseeds and various fruits and vegetables such as apples, tomatoes, citrus fruits and olives.

On May 29, 2012, the European Standing Committee advised to the Member states to collect data on the occurrence of the *Alternaria* toxins (figure 1): alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), tentoxin (TEN) and altenuene (ALT) in food commodities.

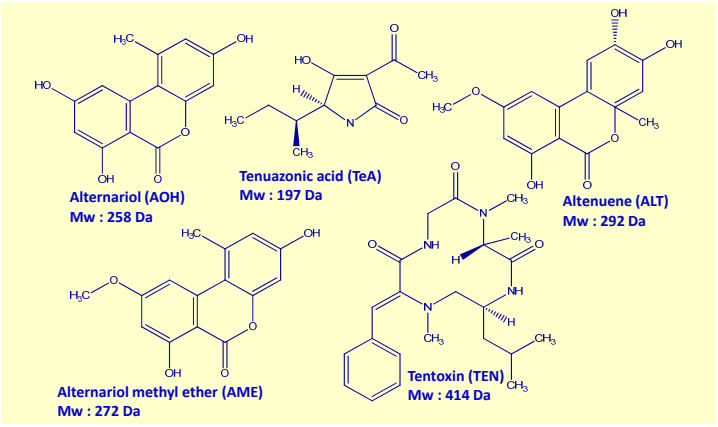

Figure 1. Chemical structure of the *Alternaria* toxins in the study.

Objective

- Adaptation of an existing LC-MS/MS method to determine 5 *Alternaria* toxins (figure 1) in various food commodities.
- Perform a survey (95 samples) to gain insight in the levels of *Alternaria* toxins in fresh apples, tomatoes, citrus, figs, olives, sunflower seeds, cereals, apple juices and tomato sauces, purchased in the Netherlands.

Results: Optimization of LC-MS/MS method

MS system: AB Sciex QTRAP® 5500

Table 1. MS/MS parameters and retention times.

	RT (min)	Transition	DP (V)	CE (V)	CXP(V)
AOH	6.90	257.0 > 215.0	-5	-30	-15
		257.0 > 213.0	-5	-26	-15
AME	7.90	271.0 > 256.0	-90	-32	-13
		271.0 > 227.0	-90	-50	-9
ALT	6.36	293.0 > 257.0	56	20	10
		293.1 > 239.1	60	30	10
TeA	6.83	196.0 > 139.0	-35	-20	-11
		196.0 > 112.0	-35	-20	-11
TEN	6.66	413.0 > 141.0	-60	-26	-11
		413.0 > 271.0	-60	-22	-11

DP: declustering potential; ; CE: collision energy; CXP: collision cell exit potential.

LC conditions: Shimadzu Prominence

- Waters Atlantis T3 3 µm, 3.0x100 mm
- T_{column}: 35 °C
- Mobile phase A: water + 1mM NH_4COOH + 1% HCOOH
- Mobile phase B: MeOH/water (96:4) + 1mM NH_4COOH + 1% HCOOH
- Flow: 0.4 mL/min

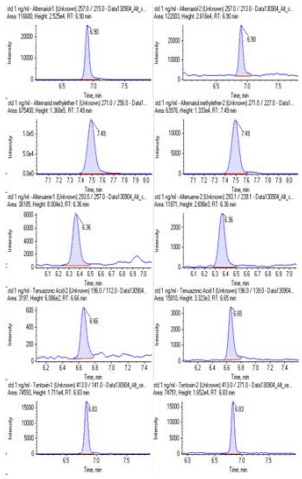

Figure 2. Chromatogram for mixed standard of 1ng/mL for AOH, AME, ALT and TEN and 5 ng/mL for TeA.

Extraction procedure

2.5 g of sample were extracted with 10 mL of acetonitrile/water/formic acid 84/16/1 (v/v/v), shaken head-over-head for 1 h and centrifuged at 3000 rpm for 10 min. 0.5 mL of the supernatant was filtered and analysed.

Results: Survey in the Netherlands

Table 2. Results of the survey for the occurrence of *Alternaria* mycotoxins.

Concentration (µg/kg)	ALT	AME	AOH	TEN	TeA
Fresh apples (N=11)					
# positives	0	0	1	0	0
Range	<1.5	<1.0	(<2.0-29)	<2.5	<5.0
Olives (N=10)					
# positives	0	0	0	0	1
Range	<1.5	<1.0	<2.0	<2.5	<5.0-5.3
Figs (N=5)					
# positives	0	0	0	0	5
Range	<1.5	<1.0	<2.0	<2.5	(25-2345)
Sunflower seeds (N=5)					
# positives	0	0	0	1	5
Range	<1.5	<1.0	<2.0	(<2.5-5.0)	(85-449)
Fresh citrus (N=11)					
# positive	0	0	0	0	0
Range	<1.5	<1.0	<2.0	<2.5	<5.0
Fresh tomatoes (N=19)					
# positive	0	0	0	0	0
Range	<1.5	<1.0	<2.0	<2.5	<5.0
Wine (N=5)					
# positives	0	0	1	0	3
Range	<1.5	<1.0	(<2.0-11)	<2.5	(<5.0-46)
Apple juices (N=7)					
# positives	0	0	0	0	0
Range	<1.5	<1.0	<2.0	<2.5	<5.0
Tomato sauces (N=8)					
# positives	0	4	4	0	8
Range	<1.5	(<1.0-7.8)	(<2.0-25)	<2.5	(66-462)
Cereals* (N=14)					
# positives	0	1	1	14	not
Range	<0.2	(<0.2-3.0)	(<0.2-5.2)	(2.5-14)	analyzed

*Analyzed by NVWA

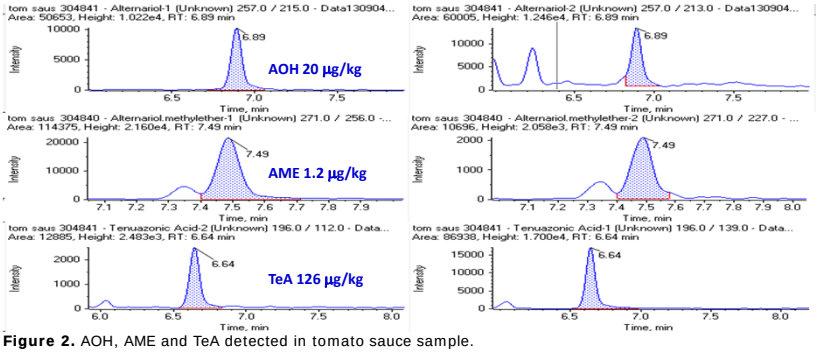

Figure 2. AOH, AME and TeA detected in tomato sauce sample.

Conclusions

- AOH, AME, TeA, and TEN were detected in one or more food commodities, while ALT was not detected in any of the samples .
- TeA was found in 27% of samples and at high concentrations.
- Regular occurrence in cereals, tomato sauces, figs, wine and sunflower seeds.
- Incidental occurrence in fresh apples, citrus fruits, tomatoes and olives.

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