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ALTERNARIA SPP. IN FOOD COMMODITIES OF SLOVAK ORIGIN: OCCURRENCE AND MYCOTOXIN PRODUCTION ABILITIES

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ABSTRACT

Various food commodities of Slovak origin were analysed for the occurrence of *Alternaria* species-groups. Totally we analysed 14 samples of grapes, 3 samples of barley, 2 samples of wheat, 17 samples of fruit, vegetable and fruit-vegetable juices, 6 samples of red kuri squash with macroscopically visible infection. Mycological analyses were performed by using plate dilution method, method of direct placing of berries or grains on the plates with dichloran, rose bengal and chloramphenicol agar or by direct inoculation by mycological needle to the identification medium (potato-carrot agar). In all grape, barley, wheat and squash samples the presence of representatives of this genus was detected (100% isolation frequency). In juices, 41% of the samples were positive for their occurrence. The highest relative density of *Alternaria* isolates was found in grape samples (87%). All detected strains were segregated into four morphological species-groups: *A. alternata*, *A. arborescens*, *A. infectoria* and *A. tenuissima*. The most dominant species-group in grapes was *A. arborescens*, in barley and wheat *A. tenuissima*, followed by *A. alternata*, in juices only *A. alternata* and *A. arborescens* species-groups were detected and isolates of squashes were not classified to the species-groups. Randomly selected 67 isolates were analysed for the ability to produce mycotoxins alternariol (AOH), alternariol monomethylether (AME) and altenuene (ALT) by means of thin-layer chromatography. Of all tested isolates, AOH production was most frequently reported (70% of tested isolates). AME was produced by 60% and ALT by 49% of tested isolates. The largest share of the productive strains originated from the squashes, where all tested isolates produced ALT and AOH, followed by isolates of juices. From the viewpoint of individual species-groups, *A. arborescens* isolates and *Alternaria* spp. appeared to be the most productive in all mycotoxins tested.

Keywords: *Alternaria* spp.; cereals; grapes; juices; mycotoxin

INTRODUCTION

Genus *Alternaria* Ness is ubiquitous, including species found worldwide in association with a large variety of substrates. Many species are saprophytes, animal/plant pathogens or postharvest pathogens **(Polizzotto et al., 2012)**. 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. They are the principal contaminating fungi in wheat, sorghum and barley. In addition to cereal crops, *Alternaria* species have been reported to occur in oilseeds such as sunflower and rapeseed, tomato, apples, citrus fruits, olives and several other fruits and vegetables. They grow at low temperature, hence they are generally associated with extensive spoilage during refrigerated transport and storage **(Ostrý, 2008)**.

In addition to spoiling a wide variety of foods, several *Alternaria* species are able to produce secondary metabolites considered as both phytotoxins, which play an important role in the pathogenesis of plants, and mycotoxins, which can be harmful to humans and animals **(Patriarca, Vaamonde and Pinto, 2014)**. *Alternaria* is one of the major mycotoxigenic fungal genera with more than 70 reported metabolites **(Escrivá et al, 2017)**. Alternariol (AOH), alternariol monomethylether (AME), tenuazonic acid (TeA), tentoxin (TEN) and altenuene (ALT) are the main *Alternaria* compounds thought to pose a risk to human health because of their known toxicity and their frequent presence as natural contaminants in food **(EFSA, 2011; Da Cruz Cabral, Fernández Pinto and Patriarca, 2016; Pose et al., 2010)**. However, food relevant *Alternaria* species are able to produce many more metabolites **(Ostrý, 2008)**, 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* fungal cultures in the search for new bioactive substances **(Patriarca, 2016)**.

Importantly, toxicological data are limited to the above mentioned major metabolites, and even these data are

incomplete, with neither good bioavailability studies nor long term clinical studies **(Andersen et al., 2015)**. 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 are also mutagenic, clastogenic, and estrogenic in microbial and mammalian cell systems and tumorigenic in rats **(Ostrý, 2008; Logrieco, Moretti and Solfrizzo, 2009)**. Some *Alternaria* mycotoxins are known for induction of DNA strand break, sphingolipid metabolism disruption, or inhibition of enzymes activity and photophosphorylation **(Escrivá et al., 2017)**. AOH and AME are mutagenic and highly active in cell based assays, but data on whole animal studies is absent in the literature **(Prelle et al., 2013)**. 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 **(Patriarca, 2016)**. Only cytotoxic activity has been proved for ALT, and TEN is a phytotoxin causing chlorosis in the seedlings of many plants **(Da Cruz Cabral, Fernández Pinto and Patriarca, 2016)**.

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 **(Patriarca, 2016)**. 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 **(Patriarca, 2016)**. According to **Andersen et al. (2015)**, viewed in food safety perspective, the food safety agencies should prioritize some *Alternaria* metabolites (specifically alternariol, alternariol monomethylether, tenuazonic acid and its derivate, tentoxin and dihydrotentoxin, altenuene, altertoxins I – III, alternarienonic acid and pyrenochaetic acid) in their monitor/observation/review programme in order to establish if *Alternaria* contamination of food and feed products constitutes a risk and if statutory guidelines should be made. Additionally, cereal and cereal products should be monitored for 4Z-infectopyrone and phomapyrone A, since these commodities also can be contaminated with strains belonging to the *A. infectoria* species-group.

The purpose of this work was therefore to monitor the occurrence of the genus *Alternaria* in various food commodities of Slovak origin and to test the ability of isolates to produce selected known toxic metabolites of this genus.

Scientific hypothesis

The *Alternaria* genus is one of the most common genera of micromyctes occurring on food commodities. Most isolates have assumptions to produce many toxic metabolites.

MATERIAL AND METHODOLOGY

Samples

Various food commodities of Slovak origin were analysed for the occurrence of *Alternaria* spp. The list and commodity origin is shown in the Table 1. Totally we analysed 14 samples of grapes, 3 samples of barley, 2 samples of wheat, 17 samples of fruit, vegetable and fruit-vegetable juices, 6 samples of red kuri squash with macroscopically visible infection. The collection of grape samples took place in the time of their technological ripeness. The grapes were picked at random by the diagonal of the land and each sample was made up of around 3 kg of grapes. Samples were collected in sterile plastic containers, stored in a cool place and transported to the mycological laboratory for analysis up to 24 hours from the collection.

Samples of barley and wheat were collected during storage, at the latest 4 months after harvest. Samples were collected in paper bags in amount of about 500 g of weight. Only grains without visible damage were used for mycological examinations.

Juices obtained from the AgroBioTech SPU Research Centre were prepared using a juicer MAGIMIX Le Duo Plus XL at room temperature 21 °C. The raw materials used for the juice production were from the Botanical Garden SPU (fruit) and from the Department of Vegetable Production FZKI SPU (vegetables). Fruits and vegetables were processed maximum within 3 hours after the harvest. Sea buckthorn berries were harvested a day in advance and frozen at -40 °C. In a frozen state it was separated from the branches, thawed and after about 3 hours pressed. Prior the processing, all fruits were washed and used vegetables were peeled, washed, sliced and pressed. The samples purchased in the trading network were 100% juices, obtained by cold pressing and treated with flash pasteurization (high temperature and short time - HTST). Juices obtained from buffets were juiced and purchased at the points of sale shown in the Table 1.

The red kuri squashes (*Cucurbita maxima*) came from one garden of domestic production grown without the use of chemicals and stored in cold rooms for maximum 1 month. During storage, some pieces of pumpkins were molded. Visibly microbiologically damaged pieces were analysed for the presence of *Alternaria* species.

Mycological analyses

Mycological analyses were performed with respect to a particular commodity. Specific ways are given in the following subchapters. In all cases (except for red kuri squashes) we used DRBC agar plates (agar with dichloran, rose bengal and chloramphenicol) **(Samson et al., 2002a)** and cultivation lasted from 5 to 7 days in darkness at 25 ±1 °C. Grown micromycetes were classified into the genera. *Alternaria* spp. were isolated by re-inoculation on the identification nutrient media PCA – potato-carrot agar **(Samson et al., 2002a)**, cultured for 7 days at room temperature and natural light and identified through macroscopic and microscopic observation in accordance with accepted mycological keys and publications **(Lawrence, Rotondo and Gannibal, 2015; Woudenberg et al., 2013; Simmons, 2007; Andersen, Kroger and Roberts, 2002; Dugan and Peever, 2002; Andersen**

Kroger and Roberts, 2001; Simmons, 1994; Simmons and Roberts, 1993).

Grapes

Grape samples were investigated for a total and endogenous mycobiota. The total mycobiota was determined by the method of direct placing of grape berries on agar plates **(Samson et al., 2002b)**. Exactly 50 berries from each sample were placed on DRBC plates**.** The endogenous mycobiota was determined by the method of direct placing of superficially sterilized berries on agar plates **(Samson et al., 2002b)**. More than 50 pieces of undamaged berries from each sample were superficially sterilized with chloramine solution, prepared from 10 mL of distilled water and 5 g of chloramine. Sterilization was carried out 2 minutes.

Grains were rinsed 3 times with sterile distilled water and dried on sterile filter paper. Exactly 50 berries from each sample were placed on DRBC plates.

Barley and wheat

The barley and wheat grain samples were analysed on exogenous and endogenous mycobiota. The exogenous mycobiota was determined by using the plate dilution method. Homogenized sample of whole grain in amount of 20 g was added to 180 mL of peptone water containing 0.02% Tween 80. Prepared suspensions were shaken on a horizontal shaker for 30 minutes. Dilutions 10^{-1}, 10^{-2} and 10^{-3} were in the triple repetition surface-inoculated in amount of 0.1 mL on DRBC agar plates.

The endogenous mycobiota was determined by the method of direct placing of superficially sterilized grains on agar plates **(Samson et al., 2002b)**. More than 100 pieces of undamaged grains from each sample were superficially sterilized with chloramine solution, in the same way as in the case of grapes. Exactly 100 grains from each sample were placed on DRBC plates.

Table 1 Overview of food commodities of Slovak origin analysed for the occurrence of *Alternaria* spp.

Food commodity	Origin	Year
Grapes - Green Veltliner	Vrbové, Small Carpathian vineyard area	2018
Grapes - Feteasca Regala		
Grapes - Chardonnay		
Grapes - Rheinriesling		
Grapes - Welschriesling		
Grapes - Sauvignon		
Grapes - Pálava		
Grapes - Pinot Blanc		
Grapes - Irsai Oliver		
Grapes - Müller Thurgau		
Grapes - Dornfelder		
Grapes - Blaufränkisch		
Grapes - Alibernet		
Grapes - Cabernet Sauvignon		
Barley 1	Štefanov	2018
Barley 2	Hrochoť	
Barley 3	Kolíňany	
Wheat 1	Kolíňany	2018
Wheat 2		
Red kuri squash	Nitra	2018
Juice - Carrot 1	Nitra, ABT RC	2017
Juice - Purple carrot		
Juice - Yellow carrot		
Juice - Beetroot 1		
Juice - White grape		
Juice - Red grape		
Juice - Apple 1		
Juice - Pumpkin		
Juice - Sea buckthorn		
Juice - Apple 2	Trademark, after flash pasteurization	
Juice - Carrot 2		
Juice - Apple 3		
Juice - Apple + beetroot 1		
Juice - Apple 4	Trnava, buffet	
Juice - Carrot 3		
Juice - Beetroot 2	Nitra, buffet	
Juice - Apple + beetroot 2		

Note: ABT RC - AgroBioTech SPU Research Centre.

Fruit, vegetable and fruit-vegetable juices

Samples were mycologically analysed within 1 hour of their preparation and the plate dilution method was used. Undiluted sample (10^0) and dilutions 10^{-1} and 10^{-2} were in two repetitions surface-inoculated in amount of 0.1 mL on DRBC agar plates.

Red kuri squashes

The squashes from which the isolates were obtained were visibly infested with filamentous microscopic fungi. The isolates were simply obtained by mycological needle from many different rotten places of the squash and multiply inoculated directly into a PCA nutrient medium. Grown micromycetes belonging to the genus *Alternaria* were subjected to mycotoxicological analyses.

Mycotoxicological analyses

For the determination of toxigenicity we used thin-layer chromatography according to the **Samson et al. (2002a)**, modified by **Labuda et Tančinová (2006)**. A total of 67 randomly selected strains of the genus *Alternaria* have been re-inoculated on yeasts extract sucrose agar (YES), cultured in the dark at a temperature of 25 ±1 °C for 7 – 14 days and then tested for the ability to produce mycotoxins alternariol (AOH), alternariol monomethylether (AME) and altenuene (ALT) by means of thin-layer chromatography. From the grown colonies we cut squares of the approximate size 2 x 2 cm and placed them into the Eppendorf tube with 0.5 mL of extraction solution chloroform : methanol, 2:1 (Reachem, SR). The content of the tubes was stirred for 5 minutes by Vortex Genie® 2 (MO BIO Laboratories, Inc. – Carlsbad, CA). The obtained extracts were applied to silica gel chromatography plate (Alugram® SIL G, Macherey – Nagel, Germany). Subsequently, we used developing solution toluene:ethyl acetate:formic acid, 5:4:1 (toluene – Mikrochem, SR; ethyl acetate and formic acid – Slavus, SR). After elution and drying, the mycotoxins have been confirmed by visual comparison with the standards of mycotoxins (ALT, AME – Merck, Germany) under UV light with a wavelength of 254 nm and 366 nm. The identity of AOH was determined on the device QTrap 4000 LC/MS/MS with TurboIonSpray ESI source and 1100 Series HPLC system. Chromatographic separation was performed at 25 ±1 °C by Gemini 5 µ C18, 150 mm x 4.6 mm (Phenomenex, USA).

Statistical analysis

The obtained mycological results were evaluated and expressed in isolation frequency (Fr) and relative density (RD) at the genus and species level. The isolation frequency (%) is defined as the percentage of samples within which the species or genus occurred at least once. The relative density (%) is defined as the percentage of isolates of the species or genus, occurring in the analyzed sample **(Guatam, Sharma and Bhadauria, 2009)**. These values were calculated according to **González et al. (1996)** as follows:

$$Fr\ (\%) = (ns / N) \times 100 \qquad RD\ (\%) = (ni / Ni) \times 100$$

Where: ns = number of samples with a species or genus; N = total number of samples; ni = number of isolates of a species or genus; Ni = total number of isolated fungi.

RESULTS AND DISCUSSION

The study focused on the monitoring of *Alternaria* spp. occurrence in various food commodities of Slovak origin, such as grapes, barley, wheat, various fruit, vegetable or fruit-vegetable juices and red kuri squashes. Analyses have shown that *Alternaria* is an important part of the mycobiota of these commodities. An overview of the occurrence of this genus is given in the Table 2. In all grape, barley and wheat samples the presence of representatives of this genus was detected. In juices, 41% of the samples were positive for their occurrence. The highest relative density of *Alternaria* isolates was found in grape samples. Also, **Swart and Holz (2017)** demostrated that the mature grape bunches were asymptomatic despite high levels of *A. alternata* recovered from triple-sterilized bunch tissue.

Isolated *Alternaria* strains were examined morphologically according to the extended keys and sporulation definitions in the identification manual by **Simmons (2007)**. Our isolates have been identified in so-called species-groups. **Simmons (1992)** defined informal species-group as a group of taxa with similar patterns of sporulation and sharing a high degree of conidial morphological characters. The species-group concept was defined, in order to simplify classification **(Patriarca, 2016)**. Following morphological analyses, strains in our study were grouped according to the colony characteristics and to their three-dimensional sporulation pattern on PCA (potato-carrot agar). All detected strains were segregated into four morphological species-groups: *A. alternata*, *A. arborescens*, *A. infectoria* and *A. tenuissima*. *A. alternata* species-group isolates were characteristic by short primary conidiophores and chains that mainly branch from the conidial body. *A. arborescens* species-group isolates formed long distinct primary conidiophores bearing branching chains of conidia. The first conidium in a branch was often longer than the others. *A. infectoria* species-group isolates were typical by short primary conidiophores and conidia in branched chains with long secondary conidiophores. They formed smooth, light coloured conidia. *A. tenuissima* species-group isolates had conidia in unbranching chains, borne on short primary conidiophores. Formation of branching conidial chains was infrequent. If branching occurred in these strains, short simple secondary conidiophores would usually originate from the conidial body.

Recent phylogenetic studies have made significant changes to the systematic taxonomy (the accurate identification of a taxon or group of taxa) within *Alternaria* by elevating 26 clades to the subgeneric taxonomic status of section **(Lawrence, Rotondo and Gannibal, 2015)**. Due to lack of molecular variation, a molecular study of **Lawrence et al. (2013)** pooled the *A. arborescens* and *A. tenuissima* species-groups with *A. alternata* into one section, called *Alternaria* sect. *Alternaria*. This section consists of approximately 60 of the common small-spored species. *A. infectoria* species-group belongs to *Alternaria* sect. *Infectoriae*, consists of

approximately 25 species **(Lawrence, Rotondo and Gannibal, 2015)**.

Grapes

Within the total mycobiota of grapes samples we recorded *A. alternata*, *A. arborescens* and *A. tenuissima* species groups. With the highest isolation frequency (100%) we recorded isolates of the *A. arborescens* species-group. They represented the largest part (48%) of all *Alternaria* isolates.

A similar representation of *Alternaria* spp. was recorded within the endogenous mycobiota. *A. arborescens* species-group occurred with the highest isolation frequency (100%) and the number of isolates represented 59% of all *Alternaria* isolates. In addition, two isolates of the

Table 2 Isolation frequency (Fr) and relative density (RD) of *Alternaria* spp. isolated from various commodities of Slovak origin.

Commodity	Analysed mycobiota	Fr [%]	RD [%]
grapes	total	100	87
	endogenous	100	81
barley	exogenous	33	3
	endogenous	100	38
wheat	exogenous	0	0
	endogenous	100	52
juices	total	41	nd

Note: nd – not determined.

Table 3 An overview of the ability of tested *Alternaria* isolates obtained from food commodities of Slovak origin to produce mycotoxins altenuene (ALT), alternariol monomethylether (AME) and alternariol (AOH) by thin-layer chromatography (TLC) – according to commodities.

Source	*Alternaria* species group	Number of tested isolates	Number/% of positive tests		
			ALT	AME	AOH
Grapes	*A. alternata*	9	1/11	1/11	1/11
	A. arborescens	15	9/60	12/80	13/87
	A. infectoria	1	0/0	0/0	0/0
	A. tenuissima	16	4/25	9/56	11/69
	∑	**41**	**14/34**	**22/54**	**25/61**
Barley	*A. alternata*	2	2/100	2/100	2/100
	A. arborescens	1	0/0	0/0	0/0
	A. tenuissima	5	3/60	3/60	3/60
	Alternaria sp.	1	1/100	1/100	1/100
	∑	**9**	**6/67**	**6/67**	**6/67**
Wheat	*A. alternata*	1	1/100	1/100	1/100
	A. arborescens	1	0/0	0/0	0/0
	A. tenuissima	4	4/100	4/100	4/100
	∑	**6**	**5/83**	**5/83**	**5/83**
Juice - Beetroot	*A. alternata*	1	0/0	1/100	1/100
Juice - White grape	*A. arborescens*	1	1/100	1/100	1/100
Juice - Red grape	*A. alternata*	1	0/0	1/100	1/100
Juice - Sea Buckthorn	*A. alternata*	1	0/0	0/0	1/100
Juice - Apple	*A. arborescens*	1	1/100	1/100	1/100
	∑	**5**	**2/40**	**4/80**	**5/100**
Red kuri squashes	*Alternaria* spp.	6	6/100	3/50	6/100
Total number of tested isolates		**67**	**33/49**	**40/60**	**47/70**

Table 4 An overview of the ability of tested *Alternaria* isolates obtained from food commodities of Slovak origin to produce mycotoxins altenuene (ALT), alternariol monomethylether (AME) and alternariol (AOH) by thin-layer chromatography (TLC) – according to isolated species-groups.

Species-group	Nr. of tested isolates	Number/% of positive tests		
		ALT	AME	AOH
A. alternata	15	4/27	6/40	7/47
A. arborescens	19	11/58	14/74	15/79
A. infectoria	1	0/0	0/0	0/0
A. tenuissima	25	11/44	16/64	18/72
Alternaria spp.	7	7/100	4/57	7/100

A. infectoria species-group were identified.

Similar results we reached in 2011, where *Alternaria* spp. colonized grapes on the surface and inside with an isolation frequency of 100%. Their relative density was 44.9% (unsterilized grapes), 57.9% (sterilized grapes). With the highest isolation frequency and relative density occurred *A. tenuissima* species-group, followed by *A. alternata* and *A. arborescens* species-groups **(Mašková et al., 2013)**.

Our grape samples were without visible growth of micromycetes, but on the other hand the authors **Kakalíková, Jankura and Šrobárová (2009)** published the first report of the *Alternaria* bunch rot on grapevines in Slovakia, which occurred during unusually hot summer weather in 2007 and 2008.

Barley and wheat

The analysis of the exogenous mycobiota of barley and wheat has produced unexpected results. On the agar plates only relatively low numbers of micromycetes have grown (from 1.4 x 10^2 CFU.g^{-1} to 4.8 x 10^3 CFU.g^{-1}). No *Alternaria* spp. were isolated from the wheat surface. On barley, *Alternaria* spp. occurred with isolation frequency 33% and only *A. tenuissima* species-group representatives were isolated.

Within the endogenous mycobiota the situation was different. The isolation frequency of *Alternari*a spp. in barley and wheat was 100%. The most common isolated species-group in both commodities was *A. tenuissima* – 61% in barley samples, 63% in wheat samples. The second most isolated species-group was *A. alternata* (more than 20% in both commodities). Less than 10% represented *A. arborescens* and *A. infectoria* species-groups. **Andersen et al. (2015)** claimed, that the *A. infectoria* species-group was unique to cereals.

Tančinová and Labuda (2009) mycologically analysed wheat bran of Slovak origin and isolated *Alternaria* spp. with frequency 62.5%. Authors detected *A. alternata* and *A. tenuissima* species-groups. **Tančinová, Kačániová and Javoreková (2001)** reported, that the low amount of fungal contamination of wheat and the high frequency of *Alternaria* occurrence suggest good storage conditions in the examined agriculture farms.

Fruit, vegetable and fruit-vegetable juices

Totally, we analysed 17 samples of different juices, of which 7 samples (41%) were positive for the presence of *Alternaria* genus isolates. All isolates were grouped into two species-groups: *A. alternata* and *A. arborescens*.

Out of 9 juices prepared in AgroBiotech, juice from yellow carrot, beetroot, white and red grapes, pumpkin and sea buckthorn were positive for the presence of *Alternaria* isolates. The best results of mycological quality were found in 4 juices purchased on the merchant network, presented as 100% cold pressed juices. These juices were heat-treated by flash pasteurization, resulting in a zero occurrence of filamentous microscopic fungi. Out of 4 juices that were produced and subsequently purchased in the buffet, only in an apple juice the presence of the *Alternaria* spp. was detected.

In a previous study **(Mašková et al., 2013)** 100% of the grape stum samples were positive for the presence of *Alternaria* genus. Relative density of this genus was 6.35%.

Red kuri squashes

A total of 6 isolates of the genus *Alternaria* were isolated from moldy squashes. In this case, due to improper storage *Alternaria* spp. caused a visible damage to the squashes. Closer identification of the isolates has not been carried out. The isolates were only tested for the production of selected mycotoxins.

Mycotoxin production

A total of 67 isolates were randomly selected for the detection of the ability to produce mycotoxins altenuene (ALT), alternariol monomethylether (AME) and alternariol (AOH) by thin-layer chromatography (TLC). The results of the analyses are processed in the Table 3. Of all tested isolates, AOH production was most frequently reported (70% of tested isolates). AME was produced by 60% and ALT by 49% of tested isolates.

Similar results have been obtained by **Andersen et al. (2015)**. The analyses in the study showed that at least 75% of the Argentinean strains are able to produce compounds (potential mycotoxins) commonly associated with *Alternaria*, such as the AOHs, altertoxins (ATXs), tenuazonic acid (TeA) and tentoxins (TENs). Less commonly produced mycotoxin was ALT (69%).

Due to the ubiquitous occurrence of *Alternaria* ssp. their mycotoxins are frequently found in a large range of foodstuff commodities. For example, AOH and AME have been detected in fruit juices **(Lau et al., 2003)**, wines **(Asam et al., 2009)** and beer **(Prelle et al., 2013)**, ALT and TeA in apple juice **(Prelle et al., 2013)**.

The largest share of the productive strains originated from the squashes, where all tested isolates produced ALT and AOH, followed by isolates of juices. On the other hand, the lowest (but not omissible) number of isolates which showed the production potential originated from grapes. The grapes from which the samples we analysed in our study were later used for wine production and according to **Zwickel et al. (2016)**, the winemaking is known to be non-effective in eliminating mycotoxins.

From the viewpoint of individual species-groups, *A.arborescens* isolates and *Alternaria* spp. appeared to be the most productive in all mycotoxins tested. An overview of the species-groups production abilities is listed in the Table 4.

Only one isolate of the *A. infectoria* species-group (from grapes) was tested and as expected, the production of the analysed metabolites has not been confirmed. The same result was recorded in previous studies **(Mašková et al., 2011; Mašková et al., 2012)**. This suggests that isolates of *A. infectoria* species-group found in food are of lesser concern than members of the *A. alternata*, *A. arborescens* and *A. tenuissima* species-groups. However, other studies have shown that some members of the *A. infectoria* species-group are able to produce altertoxin-like metabolites **(Andersen et al., 2009; Andersen and Thrane, 1996)**.

However, *Alternaria* spp. produce a variety of other metabolites for which there are no reports on function,

toxicity or if they are produced in the plants **(Andersen et al., 2015)**.

CONCLUSION

Alternaria represents an ecologically diverse fungal genus recovered worldwide as ubiquitous agents of decay of natural and artificial substrates, as confirmed in our study. Representatives of the genus *Alternaria* appeared in monitored food commodities with the high isolation frequency, especially in grapes, barley and wheat samples. *Alternaria* isolates were detected in all tested samples of mentioned commodities. The highest relative density of *Alternaria* isolates was found in grape samples (87%). All detected strains were segregated into four morphological species-groups: *A. alternata*, *A. arborescens*, *A. infectoria* and *A. tenuissima*. The most dominant species-group in grapes was *A. arborescens*, in barley and wheat *A. tenuissima*, followed by *A. alternata*, in juices only *A. alternata* and *A. arborescens* species-groups were detected and isolates of squashes were not classified to the species-groups.

In addition, the tested isolates have been shown to have a relatively high potential of the production of tested mycotoxins. Randomly selected 67 isolates produced mycotoxins alternariol (70% of tested isolates), alternariol monomethylether (60% of tested isolates) and altenuene (49% of tested isolates). From the viewpoint of individual species-groups, *A. arborescens* isolates and *Alternaria* spp. isolated from squashes appeared to be the most productive in all mycotoxins tested. However, food-relevant *Alternaria* species are able to produce many more metabolites including that known as emerging *Alternaria* mycotoxins described as potentially hazardous. Therefore, it is necessary to provide a toxicological risk assessment for agricultural products for human consumption, with regard to this genus.

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