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FILAMENTOUS MICROMYCETES RESPONSIBLE FOR THE SPOILAGE OF SELECTED VEGETABLES IN THE FOOD RETAIL CHAIN

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ABSTRACT

Vegetables represent a very important part of the diet. However, one of the problems of the vegetable market is its shelf life and related safety. A significant reason for vegetable losses in the retail network is the development of microorganisms, mainly micromycetes. They can significantly affect the quality of products, and due to the production of toxic metabolites, they also carry a toxicological risk. The aim of our study was to detect fungi that cause damage to selected types of vegetables in the food retail chains in Slovakia and are therefore directly responsible for product losses. Totally 44 samples of root, cabbage, and fruiting vegetables were mycologically analysed. Micromycetes, causing visible damage to the analysed vegetable samples, were inoculated to M137, M096, or M696 media from HiMedia and cultivated for 7 days at 25±1 °C. Important isolates were subjected to toxicological analysis by the thin-layer chromatography. The results show that the most common cause of carrot spoilage is the genus *Berkeleyomyces*. In the case of parsley, it was yeast *Geotrichum candidum*. *Alternaria* spp. occurred most often in the case of broccoli and cauliflower. Tomatoes were the most frequently spoiled by representatives of the genus *Penicillium*, or *Botrytis cinerea* and *Cladosporium*, cucumbers by *Cladosporium* and *Alternaria* species, similar to peppers, and the main spoiler of eggplants was *Botrytis cinerea*. *Alternaria* spp. showed a relatively high ability to produce altenuene, alternariol, and alternariol monomethyl ether. *Penicillium expansum* from carrots produced roquefortine C, patulin, and citrinin, and *Penicillium paneum* from tomatoes synthesized roquefortine C.

Keywords: vegetables, tomatoes, cucumber, broccoli, carrot, microscopic fungi, mycotoxins

INTRODUCTION

Vegetables are the fresh and edible parts of herbaceous plants, for example edible roots, stems, leaves, fruits or seeds. They contain valuable food components which can be effectively utilized to enhance and maintain the body's strength and repair (**Robinson, 1987**). The importance of vegetables for food and nutrition security is becoming more widely acknowledged. For the promotion of good health, vegetables are the most effective source of essential vitamins and minerals for human consumption (**Schreinemachers *et al.*, 2018**). Phytochemicals present in vegetables, especially antioxidants, also contribute to their widespread recommendation. Furthermore, vegetables are important as a source of dietary fiber (**Slavin *et* Lloyd, 2012**).
The quality of vegetables can begin to deteriorate as soon as they are cut off from their natural nutrient supply (**James *et* Zikankuba, 2017**). Even after harvesting, vegetables are living organisms that obtain their energy through the respiration process. This postharvest metabolism causes the commodity to ripen, ultimately leading to senescence. While the desirable traits that make vegetables suitable for consumption are also the same characteristics that make them vulnerable to disease, their susceptibility to illness is exacerbated during the ripening period. This commodity is prone to develop rots caused by microorganisms that hasten its ripening, internal and/or external damage, unpleasant odors, toxin production, and contamination of neighboring vegetable pieces (**Bautista-Baños, 2014**).
Microorganisms that cause spoilage can contaminate crops in various ways, such as through the seed itself, during growth in the field, during harvesting and postharvest handling, or during storage and distribution. Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (**Saranraj *et al.*, 2012**). However, colonization and lesion development more typically and more rapidly occur within damaged or otherwise compromised plant tissue. External damage such as bruising, cracks, and punctures creates sites for establishment and outgrowth of the spoilage microbes (**Watkins *et al.*, 2004**). Several types of microorganisms can thrive and grow well in many vegetables since they offer near-ideal conditions. The internal tissues of vegetables are rich in nutrients and often have a nearly neutral pH (**Miedes *et* Lorences, 2004**).
Every year, a considerable proportion of the global production of fruits and vegetables is wasted, as reported by the FAO (**Jeswani *et al.*, 2021**). Due to improper handling practices, marketing, storage problems around 20–25% of fruits and vegetables are spoilt in various stages (**Sahu *et* Bala, 2017**). Economic losses resulting from fungal infections in fruits and vegetables during the postharvest phase are not consistently documented, but typically range between 30% and 50%. On some occasions, rots can cause the total loss of the produce. Although both fungi and bacteria can be responsible for these rots, fungal infections are generally considered to be more virulent and capable of infecting a wider range of hosts throughout the entire postharvest chain (**Bautista-Baños, 2014**).
In case that food becomes spoiled due to fungi, it is typically covered in a furry growth, and takes on a soft texture, often emitting a foul odor (**Rawat, 2015**). Many serious post-harvest diseases occur rapidly and cause extensive break down of the commodity. In addition to the fact that fungi play a significant role in the spoilage of vegetables, certain health risks are also associated with their occurrence in food (**Tančinová *et al.*, 2022**). One of the important aspects is that they are considered allergenic (**Bautista-Baños, 2014**). In addition to the possibility of triggering allergic responses to dry air borne spores produced by some spoilage microbes, several spoilage molds including *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Alternaria* spp. produce toxic metabolites, called mycotoxins, which are a well-established public health hazard (**Tournas, 2005**).
The main objective of our study was to detect fungi that cause damage to selected types of vegetables in the food retail chains in Slovakia and are therefore directly responsible for product losses. Subsequently, another goal was to test potentially toxigenic isolates for the ability to produce selected toxic metabolites.

MATERIAL AND METHODS

Samples

In the first part of the work, we collected and analyzed a total of 44 samples of different types of root, cabbage, and fruiting vegetables, which were visibly damaged by the growth of microscopic filamentous fungi. As part of the root vegetables, we processed samples of carrots and parsley. From the cruciferous vegetables, we analyzed samples of cauliflower and broccoli. Finally, from fruiting vegetables we worked with tomatoes, cucumbers, zucchini, peppers, and eggplants. Specific samples and their brief characteristics are presented in Tab 1. All samples were obtained as discarded goods from the retail network in Slovakia in 2022, due to deterioration of the product by microorganisms. The samples were transported to the laboratory in their original packaging and were immediately mycologically analysed.

Table 1 Mycologically analysed vegetable samples obtained from food retail stores in Slovakia during 2022, showing signs of microbial spoilage

Nr	Commodity	Origin	Characteristics	Nr	Commodity	Origin	Characteristics
Root vegetables							
1.	Carrot	CR	washed, packed	4.	carrot	SK	washed, packed
2.	Carrot	SK	washed, packed, 1st class	5.	parsley	SK	washed, packed
3.	Carrot	SK	washed, packed	6.	parsley	SK	washed, packed
Cabbage vegetables							
Nr	**Commodity**	**Origin**	**Characteristics**	**Nr**	**Commodity**	**Origin**	**Characteristics**
7.	broccoli	PL	packed, 1st class	11.	broccoli	IT	Packed
8.	broccoli	DE	Packed	12.	cauliflower	NL	Unpackaged
9.	broccoli	PL	Packed	13.	cauliflower	NL	Unpackaged
10.	broccoli	-	Packed	14.	cauliflower	NL	Unpackaged
Fruiting vegetables							
Nr	**Commodity**	**Origin**	**Characteristics**	**Nr**	**Commodity**	**Origin**	**Characteristics**
15.	tomatoes	TN	cherry, round, 1st class	30.	cucumber	PL	green, packed
16.	tomatoes	ES	oblong, bio, 2nd class	31.	cucumber	-	Green
17.	tomatoes	PL	Bush	32.	cucumber	-	Green
18.	tomatoes	ES	cherry mix, 1st class	33.	cucumber	-	Green
19.	tomatoes	SK	cherry, 1st class	34.	cucumber	-	green, packed
20.	tomatoes	ES	oblong, bio, 2nd class	35.	cucumber	ES	Green
21.	tomatoes	NL	bush, 2nd class	36.	cucumber	ES	green, 1st class
22.	tomatoes	PL	1st class	37.	zucchini	ES	1st class
23.	tomatoes	SK	cherry, on tassel, 1st class	38.	pepper	ES	red sweet pointed, bio, 2nd class
24.	tomatoes	SK	mini, on tassel, 1st class	39.	pepper	SK	white bell, 1st class
25.	tomatoes	SK	cherry, on tassel, 1st class	40.	pepper	SK	white, PCR
26.	tomatoes	SK	cherry date, 1st class	41.	eggplant	IT	purple, globe
27.	tomatoes	SK	1st class	42.	eggplant	IT	purple, globe
28.	tomatoes	SK	plum, 1st class	43.	eggplant	ES	purple, globe
29.	tomatoes	SK	1st class	44.	eggplant	ES	purple, globe

Legend: CR – Czech Republic, DE – Germany, ES – Spain, IT – Italy, NL – Netherlands, PL – Poland, SK – Slovakia, TN – Tunisia, (-) - unknown information, characteristics - according to the available information on the packaging, or labels in the store

Mycological analyses

The grown micromycetes, causing visible damage to the analysed vegetable samples, were re-inoculated to the nutrient media MEA (Malt Extract Agar, M137, HiMedia), PDA (Potato Dextrose Agar, M096, HiMedia) or PCA (Potato Carrot Agar, M696, HiMedia), according to the presumed genera of micromycetes. Cultivation of the isolates took place for 7 days in the dark at a temperature of 25 ± 1 °C. At the same time, from mycelia grown on fruits, we prepared microscopic preparations in lactic acid with cotton-blue (**Tančinová *et al*., 2012**) and observed their microscopic characteristics.

Identification of micromycetes

Genus-level identification was based on the observation of macroscopic and microscopic features of outgrown micromycetes, according to leading mycological publications (**Samson *et al*., 2019**; **Crous *et al*., 2019**; **de Hoog *et al*., 2000**; **Pitt *et* Hocking, 1999**). We were interested in the genera spectrum of fungi that caused vegetable spoilage. Therefore, we carried out double identification, both by observing micromycetes growing directly on the vegetables and by observing isolates subsequently grown on identification nutrient media. In this way, we were able to compare the results of both identifications with each other and thus exclude the possibility that we managed to isolate a fungus on the identification medium that did not actually cause the damage.
Species-level identification of selected micromycetes was carried out using various nutrient media, according to the requirements of the given genera of fungi. To distinguish *Penicillium* species, the cultivation on MEA (Malt Extract Agar, M137, HiMedia), CYA (Czapek Yeast Extract Agar; **Pitt, 1979**), YES (Yeast Extract Sucrose Agar; **Samson *et* Frisvad, 2004**), and CREA (Creatine Sucrose Agar; **Frisvad, 1985**) at a temperature of 25 ± 1 °C for 7 days at the darkness was used. Important publications used in the identification process were **Klich (2002)**, **Samson *et al*. (2019)**, **Samson *et* Frisvad (2004)**, and **Pitt *et* Hocking (2009)**. Representatives of the genus *Alternaria* were identified into so-called species-groups based on the three-dimensional appearance of the growth of fructification structures on the PCA medium (Potato Carrot Agar, M696, HiMedia) after 7-10 days of cultivation at room temperature (21 ± 1 °C) and natural light conditions. The main identification literature was represented by publications: **Andersen, Kroger *et* Roberts (2001, 2002)**, **Woudenberg *et al.* (2013)**, **Simmons (2007)**, **Simmons (1994)**, and **Simmons *et* Roberts (1993)**.
For microscopic observation, laboratory microscopes from Olympus CX21FS1 and Olympus BX51TF with Olympus Nomarski DIC for higher contrast were used.

Evaluation of mycological analyses

The results, obtained by mycological analyses, were evaluated and expressed using the indicator called Isolation frequency (Fr), representing the proportion [%] of samples in which a particular genus or species of micromycetes occurred at least once.

Toxicological analyses

The toxinogenicity of selected potentially toxinogenic isolates was determined by thin-layer chromatography (TLC) according to the methodology of **Samson *et al*. (2002)**, modified by **Labuda *et* Tančinová (2006)**. Tested strains were re-inoculated to YES (Yeast Extract Sucrose Agar; **Samson *et* Frisvad, 2004**) and cultured in a thermostat at 25 ± 1 °C for 7-10 days. The grown mycelium of fungi with the culture medium were cut into small pieces and placed in Eppendorf tubes with 0.5 ml of extraction solution (chloroform:methanol - 2:1; Reachem, SR). The contents of the tubes were mixed for 5 minutes using the Vortex Genie® 2 device (MO BIO Laboratories, Inc. - Carlsbad, CA). The obtained fungi extracts and the standards of the monitored mycotoxins were pipetted onto a silica gel chromatography plate (Alugram ® SIL G, Macherey - Nagel, Germany). Subsequently, the plates were placed into the chromatographic vessel with the TEF mobile phase (toluene:ethyl acetate:formic acid - 5:4:1; toluene - Mikrochem, SR; ethyl acetate and formic acid - Slavus, SR), vessel was closed with a lid, and the mobile phase soared up the plate, approximately 1-2 cm from the top edge of the plate. At this stage, the plate was removed from the vessel, dried, and monitored metabolites were visualised in an individual manner.
Testing of *Alternaria* isolates was aimed at determining the ability to produce mycotoxins altenuene (ALT), alternariol (AOH), and alternariol monomethyl ether (AME). The ability of the strains to produce ALT and AME was evaluated by visual comparison of their metabolic profile with the standards of mycotoxins under UV light with wavelengths of 254 nm and 366 nm, respectively. The identity of AOH was established on the QTrap 4000 LC/MS/MS equipped with a TurboIonSpray ESI source and a 1100 Series HPLC system, with chromatographic separation performed at 25 ± 1 °C using Gemini 5 µ C18, 150 mm x 4.6 mm (Phenomenex, USA).
Isolates of potentially toxinogenic *Penicillium* species have been tested for the ability to produce metabolites patulin (PAT), citrinin (CIT), and roquefortine C (RC). The visualization of CIT took place under UV light (365 nm), and it appeared as a yellow-green spot with a characteristic tail. The visualization of PAT was done in daylight after spraying the plate with a 0.5% solution of MBTH (3-methyl-2-benzothiazolionhydrazone hydrochloride) in methanol and then heating to 130 °C for 8 minutes, after which it turned out to be a yellow-orange spot. The visualization of RC was carried out in daylight after applying $Ce(SO_4)_2.4H_2O$ to the chromatographic plate, and subsequently it appeared as an orange spot.

RESULTS AND DISCUSSION

The products of the fruit and vegetables sector which are intended to be sold fresh to the consumer are not subject to a specific marketing standard in European union and in Slovakia, too. However, this commodity must comply with the general marketing standard, listed in Regulation (EC) No 1234/2007 and Commission Implementing Regulation (EU) No 543/2011 (**European Commission, 2007, 2011**). This standard includes minimum quality requirements, minimum maturity

requirements, and defines tolerances and marking information. The products shall be intact, sound, clean, free from pests, damages, abnormal external moisture, and foreign smell and/or taste. Products affected by rotting or deterioration such as to make them unfit for consumption shall be excluded.
As mentioned in the methodological part, the analyzed vegetable samples came from retail networks, from discarded goods due to their microbial spoilage. The spectrum of analyzed vegetable species reflects the most frequently degraded and discarded species in cooperating retail chains at the time of the study. In total, 98 isolates were obtained from moldy commodity samples.

Root vegetables

Within root vegetables, a total of 6 samples were analyzed, partially 4 samples of carrots and 2 samples of parsley. Totally 5 genera of microscopic fungi, causing spoilage, were isolated from the mentioned samples, specifically *Berkeleyomyces* (formerly *Thielaviopsis*), *Fusarium*, *Geotrichum*, *Penicillium*, and *Rhizopus*. At least 2 genera of fungi were detected on each sample, the widest recorded spectrum of fungi genera (4) were found on one carrot sample. An overview of isolated microscopic fungi in individual samples shows the Tab 2. **Papoutsis *et* Edelenbos (2021)** state, that *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria radicina*, and *Berkeleyomyces* spp. are important fungi causing carrot postharvest losses and waste. According to them, fungal decay of carrots can be controlled by selecting healthy carrots and applying natural compounds, ozone, heat treatment, UV-irradiation, inorganic salt, and/or biocontrol agents and their combinations.
Carrot samples analysed in our study were processed by washing. The authors **Alegbeleye *et al.* (2022)** state in their publication that some operation, such as washing, can decrease the microbial load on vegetables. However, they may also spread spoilage microorganisms and create ideal moisture conditions that promote the growth and multiplication of these microorganisms.

Table 2 Microscopic fungi causing spoilage of root vegetables in food retail chains in Slovakia during 2022

Isolated fungal genera	Vegetable sample					
	1.	2.	3.	4.	5.	6.
Berkeleyomyces		+	+	+		+
Fusarium	+		+		+	
Geotrichum	+	+			+	+
Penicillium			+			
Rhizopus			+	+	+	

Legend: 1.-4. – carrot, 5.-6. – parsley

In carrot samples, the genus *Berkeleyomyces* was recorded with the highest isolation frequency, Fr 75%. This filamentous Ascomycete fungi is a serious root pathogen causing a disease commonly known as black root rot on many important crops such as tobacco, cotton, carrots, groundnut, and chicory (**Nel *et al.*, 2018**, **2017**). As the **Weber *et* Tribe (2004)** state, carrots, irrespective of their origin and whether they have been grown conventionally or organically, almost always harbour spores of *Berkeleyomyces basicola* and *Thielaviopsis thielavioides* on their surface. Infections become visible as dark mould patches, similarly as in our study (Fig 1), especially upon prolonged incubation in polythene bags. According to **Punja *et al.* (1992)**, **Wu *et* Huang (2015)**, and **Cavalcante *et al.* (2022)**, *Berkeleyomyces basicola* is one of the most serious diseases that impede carrot production in fields and hinders development of fresh carrot markets in many production countries. When infection is severe, mold-like lesions coalesce to cover the entire root surface, resulting in dark necrosis.

Figure 1 Carrot spoiled by a fungus from the genus *Berkeleyomyces* (in the left picture, a colony of *Penicillium expansum* is also visible)

The genus *Penicillium* was detected only in carrot samples (Fr 50%), in this case there were 2 species - *P. expansum* and *P. brevicompactum*. *Penicillium* spp. are ubiquitous soil-borne fungi and use to present in a wide range of environmental conditions (**Rodríguez-Andrade *et al.*, 2021**), many species are closely associated with human food supplies (**Pitt *et* Hocking, 2022**), such as cereals, fruits or vegetables (**Tančinová *et al.*, 2012**), and are well known for their ability to produce mycotoxins. Especially, *P. expansum* isolates produce several secondary metabolites, some of which show cellular damage, and are considered possible mycotoxins, including chaetoglobosins, citrinin, communesins, expansolides A and B, ochratoxin A, patulin, penitrem A, roquefortine C, and rubratoxin B (**Tannous *et al.*, 2018**).
Geotrichum candidum was recorded with the highest Fr in parsley samples (100%). This fungus is known to cause so called "sour rot" of root vegetables (**Horita *et* Hatta, 2016**).

Cabbage vegetables

Brassicas or crucifers are among the most significant horticultural crops worldwide, offering a broad range of products such as leafy vegetables, inflorescences, root crops, and seed crops. The quality of vegetables after harvest is influenced by both pre-harvest factors involved in their growth and the physiological state of the crop at the time of harvesting (**Hasperué *et al.*, 2019**). In the case of cabbage vegetables, 8 samples were analyzed, namely 5 samples of broccoli and 3 samples of cauliflower. In the mentioned samples, 6 genera (*Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium*, *Rhizopus*, and *Stemphylium*) were detected as spoilage agents. In general, 1, 2, or 3 different genera of fungi were isolated from individual samples. An overview of isolated micromycetes in individual samples shows the Table 3.

Table 3 Microscopic filamentous fungi causing spoilage of cabbage vegetables in food retail chains in Slovakia during 2022

Isolated fungal genera	Vegetable sample							
	7.	8.	9.	10.	11.	12.	13.	14.
Alternaria	+		+	+	+	+	+	+
Cladosporium	+		+		+			
Fusarium	+					+		
Penicillium		+						
Rhizopus								+
Stemphylium				+				

Legend: 7.-11. – broccoli, 12.-14. – cauliflower

In both types of vegetables (Fig 2), the genus *Alternaria* occurred with the highest Fr, with values of 80% in broccoli and 100% in cauliflower. We noticed a difference in the species-groups spectrum of this genus. In broccoli, *A. tenuissima* species-group dominated, as it was present in 3 samples solo and in one sample together with *A. brassicicola*, and one different but unidentified strain, marked as *Alternaria* sp. In cauliflower, only isolates of the *A. brassicicola* species-group were detected. Another frequent fungus on broccoli was the genus *Cladosporium* (Fr 60%). The genus *Penicillium* in broccoli was represented by the species *P. olsonii*.
Endemic disease caused by *Alternaria* spp., called "Alternaria blight" is one the most devastating diseases of broccoli, which occurs in all parts of the world where broccoli is grown (**Kabir et al., 2013**), and infections by this fungus cause severe yield losses to those crops. Cauliflower growers also encounter a similar problem, while *Alternaria* spp. can cause up to 80% yield reduction (**Kashyap *et* Dhiman, 2010**). Phytopathogens from *Alternaria* genus are known to synthesize phytotoxins that damage plant tissues and facilitate colonization (**Pedras *et al.*, 2011**).

Figure 2 Broccoli and cauliflower contaminated with *Alternaria* spp.

The broccoli samples, mycologically analysed in this study, were in the retail chains tightly wrapped in a foil. It is possible that this method of packaging may, under certain circumstances, promote the development of micromycetes. According to **Artés *et* Martínez (1999)**, the use of various polymeric films to wrap products is a common method for reducing water loss, which can help maintain firmness and prevent wilting. However, this practice can also lead to increased decay, potentially caused by the buildup of free water on both the surface and within the package.

Fruiting vegetables

Fruiting vegetables were represented by the highest number of samples (30), namely 15 samples of tomatoes, 7 samples of cucumbers, 1 zucchini, 3 samples of peppers, and 4 samples of eggplants.

Tomatoes are among the most widely produced and highly consumed vegetable crops in the world (**Grandillo *et al.*, 1999**). After they are harvested, tomatoes quickly lose their quality due to their highly perishable nature. Therefore, it is crucial to implement effective postharvest handling practices, including controlling temperature and humidity levels, regulating gas composition during storage, applying calcium chloride, and employing proper physical handling techniques, in order to preserve their quality (**Arah *et al.*, 2015**). Due to their higher water content, tomatoes are particularly vulnerable to spoilage by fungi (**Samuel *et* Orji, 2015**). In our study, a total of 9 fungal agents of spoilage were isolated from tomatoes (Fig 3) – *Alternaria*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Geotrichum*, *Mucor*, *Penicillium*, *Rhizopus*, and *Stemphylium*. In the study, minimum 1 fungus and maximum 4 fungi in one sample were recorded. Table 4 provides an overview of the fungi isolated from the samples.

Table 4 Microscopic fungi causing spoilage of fruiting vegetables (tomatoes) in food retail chains in Slovakia during 2022

Isolated fungal genera	Vegetable sample														
	15.	**16.**	**17.**	**18.**	**19.**	**20.**	**21.**	**22.**	**23.**	**24.**	**25.**	**26.**	**27.**	**28.**	**29.**
Alternaria		+				+			+				+		
Botrytis	+	+	+	+			+					+			
Chaetomium				+											
Cladosporium		+		+		+		+	+				+		
Geotrichum								+					+		
Mucor													+		
Penicillium	+		+	+	+		+		+	+	+	+			
Rhizopus														+	+
Stemphylium		+				+									

Legend: 15.-29. – samples of analysed tomatoes

Based on the results, we can consider the *Penicillium* isolates to be the most frequent (Fr 60%) and therefore the most important fungi in spoiling of this commodity. The species *P. brevicompactum*, *P. glabrum* clade, *P. hoeksii* clade, *P. olsonii*, *P. paneum*, and *P. polonicum* were isolated from individual samples. Out of these species, *P. olsonii* was clearly the most dominant and represented 61.5% of all *Penicillium* isolates. All strain were isolated from cherry tomatoes (on tassels or bushes). Other *Penicillium* species represented 7.7% each.
The report of *P. olsonii* causing postharvest fruit rot on tomato in Serbia is given by **Živković *et al.* (2021)**. Previously it was also reported on tomato fruit in Canada (**Chatterton *et al.*, 2012**) and Pakistan (**Anjum *et al.*, 2018**). Authors described the infection, as the small circular, water-soaked lesions with white mycelia on the outer layer of the affected area, which then progressed to form extensive watery rot and larger gray lesions.

Figure 3 Tomatoes contaminated with various micromycetes

Botrytis cinerea and *Cladosporium* spp. were also present in tomato samples with a significant Fr (both 40%). *Alternaria* isolates were present with a Fr 27%, and its isolates included *A. arborescens* and more often *A. tenuissima* species-groups. Similarly, in the study of **Andersen *et* Frisvad (2004)**, members of the *A. tenuissima* species-group were found to be the most dominant fungi on moldy tomatoes. According to **Moss (2008)**, *Alternaria* spp. are able to grow on a wide range of fruits and vegetables and are a major pathogen of fresh tomatoes in which they can produce tenuazonic acid. The absence of evidence linking tenuazonic acid or other *Alternaria* metabolites to human illness is reflected by the fact that no regulatory limits have been established for them.
From other types of fruiting vegetables, 6 genera of microscopic filamentous fungi were isolated (*Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Rhizopus*). Either 1 or 2 spoilage agents were detected in each sample. Table 5 shows an overview of micromycetes isolated from these commodities.
Mycological analysis shows that the *Cladosporium* has a relatively high affinity for cucumbers and zucchini. This fungus is very cosmopolitan in distribution and is commonly encountered on all kinds of plants (**Bensch *et al.*, 2012**). *Cladosporium* species are well known as pathogens on variety of fresh fruits and vegetables (**Pitt *et* Hocking, 1999**). From cucumbers, *Cladosporium* spp. were isolated with the highest isolation frequency (71%), followed by *Alternaria* spp. (57%), represented by *A. arborescens*, *A. infectoria*, and *A. tenuissima* species-groups. *Alternaria* spp. (especially strains of *A. tenuissima* species-group) were also isolated from peppers of Slovak origin. As stated by **Tournas (2004)**, many fungal genera show a specific substrate preference, but fungi such as *Alternaria*, *Botrytis*, *Cladosporium*, *Rhizopus*, and some others show the ability to unlimitedly attack a wide variety of vegetables, causing devastating losses.
Among the *Penicillium* genus, only 2 strains were isolated, namely *P. dipodomyis* from cucumber and *P. olsonii* from eggplant. However, *Botrytis cinerea* appears to be a more significant cause of eggplant losses in retail chains (Fig 4), as all analyzed fruits were attacked by this pathogen. In all cases (as can also be seen in Figure 4), the fungal infection spread from the calyx. Similar findings had **Temkin-Gorodeiski *et al.* (1993)**, who reported that the eggplant fruits deteriorate rapidly during prolonged storage, mainly due to accelerated senescence of the calyx. As regards the causative agent of the spoilage itself, **Moss (2008)** stated that a typical feature of the *Botrytis cinerea* is, that it has a wide host range, and may cause spoilage of many fruits as well as vegetables, indeed it is reported to be pathogenic to more than 200 species of plants. Moreover, *Botrytis* possesses multiple cutinases and lipases that are capable of degrading plants rich in pectin (**van Kan, 2006**).

Table 5 Microscopic filamentous fungi causing spoilage of fruiting vegetables (except tomatoes) in food retail chains in Slovakia during 2022

Isolated fungal genera	Vegetable sample														
	30.	**31.**	**32.**	**33.**	**34.**	**35.**	**36.**	**37.**	**38.**	**39.**	**40.**	**41.**	**42.**	**43.**	**44.**
Alternaria	+	+	+	+						+	+				
Botrytis									+			+	+	+	+
Cladosporium	+		+	+	+	+		+		+					
Fusarium					+			+							
Penicillium						+							+		
Rhizopus							+								

Legend: 30.-36. – cucumbers, 37. – zucchini, 38.-40. – peppers, 41.-44. - eggplants

Figure 4 Eggplant and red pepper contaminated with *Botrytis cinerea*

Toxicological analyses

In the following section, attention was paid to isolates of *Alternaria* and *Penicillium* genera, which are known for their toxigenic properties. In Slovakia, as well as in the European Union, there are no decrees regulating the toxin content of fresh (unprocessed) vegetables. From the previous findings, it follows that *Alternaria* genus had the highest representation in cabbage vegetables, although a significant occurrence was also recorded in peppers, cucumbers, and tomatoes. The genus is associated with the biosynthesis of a diverse range of secondary metabolites, many of which have biological activity (**Moss, 2008**). In total, 4 species-groups of this genus were identified on vegetables in this study - *A. brassicicola*, *A. arborescens*, *A. infectoria*, and *A. tenuissima*. All of them, with the exception of *A. infectoria*, are considered potential producers of many known mycotoxins (**Mašková *et al.*, 2019**; **Andersen *et al.*, 2002**; **Adama *et al.*, 2021**). *Alternaria infectoria* has a markedly different metabolic profile, and the production of the mentioned metabolites by isolates of this species-group has not been confirmed yet. The strains of this species-group also differ macromorphologically from the strains of other species-groups, such as *A. brassicicola*, *A. arborescens*, or *A. tenuissima*. *Alternaria infectoria* forms a lighter, grey-white mycelium, as the other groups are rather darker olive green to brown or almost black. The inability to produce known mycotoxins can additionally be used as an auxiliary identification feature in the case of "light-color" *Alternaria* strains. In our study, only one isolate of the *A. infectoria* species-group was isolated, and its ability to produce tested mycotoxins (altenuene – ALT, alternariol – AOH, and alternariol monomethyl ether – AME), as we assumed, was not confirmed. The production abilities of our remaining 22 strains are summarized in Tab 6. The table shows a relatively high ability of our isolates to produce tested metabolites. With the exception of one strain (*A.* sp. from broccoli), all tested isolates produced at least 2 of analysed mycotoxins. Overall, we can say that 77% of the strains produced ALT, 82% synthesized AOH, and 91% AME.

Natural occurrences of AOH, AME, and in some cases other *Alternaria* toxins have been reported in various food commodities of plant origin, including tomatoes, tomato products, and peppers (**Scott, 2001**; **Fraeyman *et al.*, 2017**). An important toxic metabolite of this genus is also tenuazonic acid, although analyzes of its production were not included in our study, but its production is often recorded together with the metabolites AOH, AME, and ALT (**Andersen *et* Frisvad, 2004**). Despite the frequent detection of these mycotoxins, there is currently no legislation or guidelines in place to regulate their presence, and limited *in vivo* toxicity data is available (**Fraeyman *et al.*, 2017**).

Table 6 The ability of *Alternaria* spp., causing spoilage of various types of vegetables, to produce selected mycotoxins, determined by the method of thin-layer chromatography (TLC)

Commodity	**Species-group**	**Mycotoxin production ability (number of positive/tested strains)**		
		ALT	**AME**	**AOH**
broccoli	*A. brassicicola*	0/1	1/1	1/1
	A. sp.	0/1	0/1	0/1
	A. tenuissima	4/4	3/4	4/4
	Σ	**4/6**	**4/6**	**5/6**
cauliflower	*A. brassicicola*	**3/3**	**2/3**	**3/3**
tomatoes	*A. arborescens*	1/2	2/2	1/2
	A. tenuissima	4/5	5/5	5/5
	Σ	**5/7**	**7/7**	**6/7**
peppers	*A. tenuissima*	**2/2**	**1/2**	**2/2**
cucumbers	*A. arborescens*	2/2	2/2	2/2
	A. tenuissima	1/2	2/2	2/2
	Σ	**3/4**	**4/4**	**4/4**
Totally		**17/22**	**18/22**	**20/22**

Legend: ALT – altenuene, AOH – alternariol, AME – alternariol monomethyl ether, *A.* – *Alternaria*

The genus *Penicillium* was the most frequently found on tomatoes, where the highest species diversity was also recorded. In the presented study, a total of 8 species of this genus were isolated from vegetables – *P. brevicompactum*, *P. dipodomyis*, *P. expansum*, *P. glabrum* clade, *P. hoeksii* clade, *P. olsonii*, *P. paneum*, and *P. polonicum*. Out of the listed species, *P. expansum* and *P. paneum* are both considered to be a potential producer of some important metabolites. According to the literature, *P. expansum* is capable of several compound synthesis, including citrinin, ochratoxin A, patulin, penitrem A, roquefortine C, and rubratoxin B (**Andersen *et al.*, 2004**). In our case, *P. expansum* isolated from carrots, was tested for the roquefortine C – RC, patulin – PAT, and citrinin – CIT production. Our strain produced all three metabolites. Regarding *P. paneum* species, the synthesis of mycotoxins such as RC and PAT is known (**Boysen *et al.*, 1996**). Our strain from tomatoes was analysed for the production of these metabolites, and only synthesis of RC was confirmed.

CONCLUSION

Vegetables play an important role in providing essential nutrients, dietary fiber, and phytochemicals that promote good health. Despite their nutritional benefits, vegetables are susceptible to rapid quality deterioration after being harvested, mainly due to the respiration process and vulnerability to microorganism-induced diseases. Food spoilage caused by microorganisms is a worldwide problem that leads to both food waste associated with economic losses and dissatisfaction among customers. Microscopic filamentous fungi find suitable conditions for growth on many types of vegetables, and as confirmed in our study, many times they can produce a wide range of toxic metabolites. The detection of potentially toxigenic isolates and their ability to produce selected mycotoxins highlights the importance of proper handling practices and storage to minimize fungal contamination and ensure the safety and quality of vegetables. Some important measures may include harvesting at the right time, handling with care to avoid damages, washing and drying, proper storing, regular monitoring during the storage and possibly consideration of some natural antifungals. Proper temperature management immediately after harvesting is a crucial factor in prolonging the shelf-life of vegetables, and indirectly preventing the loss of quality characteristics during postharvest storage.

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