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Ecological and sanitary significance of micromycetes brought from abroad with various foodstuffs of floral origin

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During 2003–2006, micromycetes recorded on a variety of raw materials and foodstuffs of plant origin brought into Lithuania and sold here were investigated; their species composition was determined, their abilities to synthesize and excrete secondary metabolites and spread in the environment were studied. Many micromycetes brought together with raw materials and foodstuff are able to adapt to new ecological conditions, penetrate into commercial, industrial and residential premises. They enrich the species diversity of local micromycetes, extend their functional, destructive abilities, which are not always favourable to people; they can also cause economic losses as well as be hazardous to the health of man, as some micromycetes can cause diseases of respiratory organs, be causative agents of allergies, skin diseases, or even deep mycoses of some organs. Toxic secondary metabolites excreted by microorganisms worsen the quality of food, cause permanent hazard to its safety. Basing on the research results, the above-mentioned issues are discussed in the article.

Key words: fungi, contamination, toxin, vegetables, fruit, grain, products, imported

INTRODUCTION

Nowadays industrial and commercial relations are rapidly expanding. From various distant countries, where ecological conditions are different from those in the temperate climate zone, a vast majority of foodstuffs of plant origin are imported; they enrich the nutrition of people with valuable substances, add variety to food flavour, provide people with positive emotions and have a great economic importance. Simultaneously, together with the imported raw materials (grain, fruit, vegetables, seeds, berries and their products) microorganisms that intersperse the diversity of the local micromycetes are brought. The newly brought micromycetes start to spread, sometimes supersede local species, sometimes intercross with them and function further, thus changing the ecological features of the environment; production conditions of raw materials and their products change; new problems of food safety arise. It is confirmed by the research performed by a number of specialists using various objects (Adeyanju, Ikotun, 1988; Lacey, 1988; Lugauskas, 1988; Kapat et al., 1998; Birzell et al., 2000; Elen et al., 2000; Gherbawy, Prillinger, 2000; Dutkiewicz et al., 2001; Krysinska-Traczyk et al., 2001; Bottalico, Perrone, 2002; Klich, 2002; Huttunen et al., 2004).

Many micromycetes detected on the imported food products are potential producers of toxic secondary metabolites (Fanelli et al., 1981; Кудряшева, 1986; Bennett, Bõrjesson et al., 1999; Elmholt, Hestbjerg, 1999; Simsekli et al., 1999; Cole, Schweikert, 2003; Cole et al., 2003; Klich, 2003; Riteni, 2003; Anderson et al., 2004).

According to Hungarian researchers (Fazekas et al., 2002), grains of all cereals as well as coffee beans are heavily contaminated with micromycete propagules. Basing on Frisvad (1988), however, the specificity of toxic metabolites is determined by the peculiarities of micromycete species as well as the environment of the toxin synthesis.

The specificity of the produced toxins is determined by the connection between penicillia and aspergillia and mycotoxins, with a special emphasis on misidentified isolates (Frisvad, 1989; Frisvad, Thrane, 2002). Similar results were obtained after analysis of the relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurrence on *Argentinian durum* wheat (Gonzàlez et al., 1999). It should be noted that fungi of the genus *Aternaria* synthesize toxins even in cases when they are isolated from firm green apples or ripening vegetables (Gvinyn, Szteke, 1995; Robiglio, Lopez, 1995). The fungi of the

genus *Fusarium* are able to synthesize diverse toxins. It has been confirmed by the research of Finnish scientists on *Fusarium* micromycetes damaging various crops, performed in 1998–2000 (Jimenez et al., 1997; Yli-Mattila et al., 2000). A research performed in Lithuania in 1990–1999 together with Norwegian researchers has shown that *Fusarium* fungi strongly deteriorate the nutritive value and realization potential of grains (Keblys et al., 2000). The *Fusarium* fungi, active producers of toxins, cause serious problems to the growers and consumers of corn (Ueno et al., 1986; Mills, 1990; Pieckową, Jesenska, 2001; Lugauskas, Krasauskas, 2005). Micromycetes of the genus *Penicillium*, which in Lithuania are widely distributed in soil and other substrates, produce toxic secondary metabolites of a very diverse chemical composition. Micromycetes of this genus are also abundant on imported food products (Лугаускас, 1988; Винокурова et al., 1991, 1993; Осипян, Батикян, 1993; Решетилова и др., 1993; Tomšiková, 1994; Veselà, Veselý, 1994; Petersson et al., 1998; Ross et al., 1998; Christensen et al., 1999; Elmholt, Hestbjerg, 1999; Lugauskas et al., 2002, 2005; Anderson et al., 2004). Some micromycetes of this genus are resistant to high temperature, therefore, they are often detected on freshly dried or otherwise treated fruits, vegetables, seeds, grain (Jesenska, Pieckova, 1995; King, 1997; Wareing, 1997). Thus, the statement of H. Kamimura (1988) concerning the removal of mycotoxins during food processing is not always true. On plants cultivated and naturally growing in Europe, which are more or less used for food, plenty of parasitic fungi are detected (Brandenburger, 1985); many of these fungi are able of producing toxic secondary metabolites and use them to impair the immune system of their hosts. Mycotoxins can accumulate in all plant organs and, therefore, get into the environment of people or even on their tables with fruits, berries, seeds, grain ((Wouters et al., 2000). The ability of fungi to synthesize mycotoxins is determined by water activity, temperature, light, substrate specificity and other environmental factors (Cuero et al., 1987). The ability to synthesise toxic secondary metabolites, as well as the degree of pathogenicity, is often predetermined by the origin of micromycetes (Khan et al., 1988). Many factors influence the composition of the produced toxic secondary metabolites and consequently their impact upon the environment. Some micromycetes of the genera *Aspergillus* and *Penicillium* synthesize nitrogen-rich compounds that disturb the mental health of humans and animals, others produce compounds inducing the reproduction of cancerous cells, still others produce compounds causing functional disorders of one or several organs (Kriek, 1988; Niyo et al., 1988; Козловский, 1996; Rio et al., 1997; Schnürer et al., 1999; Pfohl-Leszkowicz et al., 2002; Walker, 2002). Micromycetes penetrating into the technological processes of food production should be also mentioned. In this way, the normal technological course is broken and the raw materials and final products become contaminated with undesirable toxic secondary metabolites (Scholte, Samson, 2000; Samson et al., 2002). Fungi of the genera *Rhizopus* and *Neurospora*, which while growing in peanuts are able to accumulate aflatoxin B_1 synthesized by other fungi (*Aspergillus flavus* and *A. parasiticus)*, have been determined (Nout, 1989). It is urgent to determine contamination of imported fruit, vegetables, berries, seeds, grain with micromycete propagules, therefore, the existing methods are being applied and new methods for determination of mycotoxins in raw materials and products are searched for (Milanez, 1995; Nielsen, Smedsgaard, 2003); methods for identification of the producers of toxic secondary metabolites are elaborated, because the morphological and cultural properties of micromycetes brought from other countries often differ from those of local strains (Kuhls, 1999; Kulling et al., 2000).

To protect food from contamination with mycotoxins, it is essential to investigate the producers, ways of their access to food, reduce the possibilities of their spread and functioning in the environments of food processing, storing, realization and consumption.

The aim of the research was to determine micromycetes on raw materials of floral origin imported to Lithuania from various countries, to identify their systematic position and potential abilities to produce and excrete toxic secondary metabolites, to check the possibilities of their accumulation in food and potential risk to the health of consumers.

MATERIALS AND METHODS

Premises. During the period 2003–2006, samples of imported vegetables, fruit, berries, seeds, grains and their products were taken in two wholesale centres to which many trading companies supply imported goods. Part of the imported goods was packed in places of their growth or in storehouses of the countries of their origin and, therefore, the origin of their fungal infection is ascribable to the ecological niche of that environment. Another part of production during transportation was in contact with the ambient air, transportation vehicle and got into new storehouses with certain mycological conditions. The imported products were stored, distributed, transported and marketed in a new environment. During these processes the possibilities for the products to get contaminated with micromycetes present in the premises occurred. As in large wholesale centres there is an intense movement of people and vehicles, micromycete propagules can easily get on the products from the outdoor air and environment. The surface of the majority of dried fruit and vegetables is sticky. It helps micromycete propagules attach to the surface of products and start their functioning. In both wholesale centres the imported raw material of plant origin and products (vegetables, fruit, berries, seeds, processed and unprocessed grains) were on sale.

Isolation of micromycetes. In the above-mentioned premises, 760 samples of vegetables, fruit, berries and

other foodstuffs of plant origin were taken. They were analyzed according to the methods described by Samson et al. (1992), Pitt (1997), Rabie et al. (1997), Lugauskas, Stakėnienė (2002), Mathur, Kongsdal (2003), Lugauskas et al. (2005). When visual observation allowed the presumption that the sample was contaminated by one infection agent, the method of plating was employed, and in cases of possible mixed infections the method of diluting was applied. In the first case, a piece of an infected product, cut off with a sterile scalpel, was placed onto a Petri dish containing malt extract agar medium with chloramphenicol (50 mg/l). In the second case, 1 g of a product was taken and placed in 100 ml of sterile water, shaken for 15 min, and a series of dilutions were done. From each dilution series, 1 ml of suspension was drawn into a 9 cm diameter Petri dish and poured over with 15 ml (48 °C) of the same malt extract agar medium enriched with an antibiotic. The dishes were kept for 4 days in a thermostat at a temperature of 28 °C and for the next 4 days at 20 °C; the light and dark regime was changed every 12 hours. Pure micromycete cultures were isolated, cultivated in standard Czapek agar, standard malt and corn extract media at a temperature of 28 °C for 5–7 days and identified. Detection frequency (%) of each identified species was calculated.

Evaluation of micromycete toxicity. Ability of micromycetes to synthesize and excrete toxic secondary metabolites was tested applying methods described by Frisvad (1988), Betina (1991), Smith et al. (1995), Raudonienė, Lugauskas (2005). Micromycetes were cultivated on standard Czapek, Czapek yeast agar (CYA) and yeast extract – sucrose agar (YES) media for 7–14 days at a temperature of 28 °C. Significant changes in the color of fungal colonies and abundant excretion of pigment into CYA and YES media, to compare with the growth on standard Czapek media, in the authors' opinion allow to suppose that the study strains can be potential producers of mycotoxins. The above-mentioned media particularly induce the synthesis of mycotoxins by micromycetes of the genera *Aspergillus, Penicillium, Fusarium* and *Alternaria*. Primary selection of micromycetes according to their ability to synthesize toxic metabolites was performed employing the method of thin-layer chromatography (ISO standard 8178-2: 1999). The following systems of solvents were used in the study: chloroform – methanol (98:2), chloroform – methanol (8.5:1.5), chloroform – methanol (95:5), toluene – ethyl acetate-formic acid (5:4:1), benzol – acetic acid (3:1), toluene – acetone – methanol (5:3:2).

Determination of toxins in samples of vegetables, fruits, berries, seeds, grain and their products was performed by the ELISA method (Samson et al., 1992; Smith et al., 1995). Extraction of mycotoxins and tests were performed according to manufacturers' instructions. The VERATOX®, Alatox (total), VERATOX® DON 5/5, VERATOX® Ochratoxin, Aflatoxin, T-2 toxin, zearalenone and RIDA CHREEN® Ochratoxin A test kits (R-Biopharm AG, Germany) were used for the analysis.

Statistical analysis. The obtained results were processed using Microsoft Excel 2000, Statistica 5.1 software.

RESULTS AND DISCUSSION

Vegetables prevailing on the market in Lithuania and brought from Poland, Spain, Holland and other countries were investigated. Carbohydrate-rich potatoes are among most frequently and abundantly consumed vegetables; they are used in industry for the production of starch and in agriculture for animal fodder. It has been previously determined that from the stored potatoes grown in Lithuania, micromycetes widely distributed in soil are most frequently isolated. Their propagules together with soil particles get into the storehouses and under favourable conditions start developing thus causing rot, especially of mechanically injured potatoes. From injured potato tubers grown in the country and imported from Poland and Holland, *Acremonium strictum* (detection frequency over 11%), *A. charticola* (7%), *Mortierella hyalina* (9%), *Fusarium oxysporum* (5%), *F. solani* (7%), *Trichoderma harzianum* (4%), *Geotrichum candidum*, *Rhizopus oryzae*, *Sclerotinia sclerotiorum* (over 2%) were frequently isolated. In the wholesale potato storehouses, depending upon technological conditions of storing, the composition and abundance of micromycete species varied. In some storehouses, potatoes were damaged by *Verticillium alboatrum* (up to 50%), *Gliocladium deliquescens*, *Fusarium equiseti* (about 33%), higher amounts of potatoes were damaged by *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (detection frequency reached 66%). *Mucor circinelloides*, *Fusarium oxysporum, F. merismoides* fungi were recorded. In the premises of short-term storage and sorting, where washed potatoes from Spain were stored, micromycetes were not abundant; *Acremonium potronii*, *Cladosporium cladosporioides*, *Fusarium anthophilum*, *Penicillium piscarium* were isolated. On imported potatoes, various micromycetes of the genera *Penicillium* and *Aspergillus* were recorded, but not abundantly. These are causative agents of secondary infection. More frequent were *Penicillium expansum* (detection frequency over 11%), *P. funiculosum*, *P. lanosoviride*, *P. spinulosum* (about 9%), *P. variabile* (over 4%), *Aspergillus fischeri*, *A. niger* (about 7%), which are able to excrete toxic secondary metabolites. From imported potatoes: *Acremonium roseum*, *Fusarium solani*, *F. equiseti*, *Gliocladium catenulatum*, *G. deliquescens*, *Chrysosporium merdarium*, *Sclerotinia fuckeliana*, *Sporotrichum aurantiacum*, *Galactomyces geotrichum*, *Mucor racemosus*, *Phoma exigua*, *Rhizopus oryzae* micromycetes were isolated, but their propagules were not abundant.

In *Penicillium verrucosum* alcohol biomass extract, in the system of solvents toluene-ethyl acetate-formic acid (5:4:1), there were 6 compounds forming dark

purple fluorescence in the presence of UV light (Fig. 1). The compound with Rf 0.60 was identified as patulin, on comparison with a standard.

Tomatoes, paprika, aubergines belong to the same family as potatoes. The parts of these plants that are used for food do not contact with soil, however, micromycetes get on them from the air, packing containers or hands of people; soil particles get on them together with dust which abounds in micromycete propagules. The chemical composition of these vegetables is different than that of potatoes, i.e. they are watery and vitamin-rich. Therefore, the species composition of micromycete species infecting them is different. *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* with the detection frequency 25% were isolated from paprika grown and sold in various localities. On paprika imported from Spain, *Botrytis cinerea, Geotrichum fermentans, Mucor* spp., *Penicillium chrysogenum*, *Radiomycopsis embreei* were recorded. In supermarkets, Spanish tomatoes are intensively damaged by *Rhizopus oryzae* (90%), less frequently by *Alternaria solani* (66.7%), *Penicillium granulatum* (50%), which cause black rot. On aubergines imported from Spain, many *Penicillium* species were recorded (*P. cyaneofulvum*, *P. decumbens*, *P. expansum*, *P. oxalicum,* etc.). Some of them are active producers of patulin, roquefortine, cyclopenin, chaetoglobosin C, citrinin, oxaline and other mycotoxins.

Another important group of vegetables used for food belong to the family of umbelliferous plants. These plants are characterized by the synthesis of volatile oils and provitamins; they are rich in cellular tissue which improves digestion processes, activate persitalsis. Carrots are most widely used; they are usually grown in Lithuania, sometimes are imported from the neighbouring countries. In samples of carrots (taken in the premises of short-term storage, sorting and selling) which had been imported from Poland, conditionally pathogenic micromycetes of the *Fusarium equiseti*, *Verticillium tenerum* species together with *Absidia blakesleeana*, *Mucor hiemalis*, *M. mucedo,* ascribed to cosmopolitan species, were recorded. Sometimes *Tilachlidium brachiatum* fungi were detected; the issue of their systematic position is still under discussion, and data on their ecological and physiological peculiarities are scarce.

Acremonium strictum, *Mortierella polycephala*, *Penicillium claviforme*, *P. expansum, P. italicum* fungi were frequently isolated from imported parsnips, root parsley, celery. Celery tubers were damaged by *Verticillium alboatrum* (detection frequency reaching 40%). On dill sprays *Acremonium strictum* (about 50%), *Penicillium digitatum* (33%), yeast-like fungi dominated, sometimes *Cladosporium cucumerinum* fungi were isolated; they are usually considered as the agents of cucumber pox.

On cruciferous plants (Japanese radish, small radish, radish grown in Lithuania and Poland), *Acremonium strictum, Cladosporium cladosporioides, Fusarium oxysporum* (detection frequency about 40%) dominated; less frequent were *Sclerotinia sclerotiorum*, *Verticillium alboatrum*. Radish were frequently damaged by *Fusarium solani* (75%), *Penicillium paxilli* (50%) fungi. *Mucor lusitanicus* (= *Mucor racemosus* var. *lusitanicus*) fungi were isolated from Japanese radish with the detection frequency about 50%. Cauliflowers on sale in the markets of Vilnius, whatever their origin, were most frequently damaged by *Aspergillus niger* fungi. In storehouses, plenty of cabbages are damaged by *Botrytis cinerea* (about 43%), *Peronospora brassicae* (up to 29%), *Sclerotinia sclerotiorum* (up to 43%); active producers of toxins *Penicillium claviforme, P. expansum, P. granulatum* are also frequent, together with *Absidia glauca*, *Mucor hiemalis*, *M. racemosus*, *Trichoderma aureoviride* and other micromycetes. From cabbages on sale in the market place in Vilnius, *Piptocephalis lemonnieriana* fungi were isolated for the first time in Lithuania. However, the knowledge of their ecological and physiological peculiarities is still insufficient. Rather frequently cabbages grown in the country and imported from abroad were damaged by *Alternaria alternata*, *A. brassicicola*, *Sclerotinia sclerotiorum* (detection frequency about 67%), *Cladosporium herbarum* (33%) fungi. The sources of their infection are hardly detectable.

Vegetables of the cucurbit family usually contact soil; therefore typical soil fungi are frequent on them. The skin of young cucumbers, zucchini, marrow, squash is thin and juicy and thus easily damaged by *Alternaria alternata* (70%), *Botrytis cinerea* (40%); later *Mucor murorum*, *Penicillium meleagrinum*, *Cladosporium cucumerinum*, *Aspergillus fumigatus, Gliocladium catenulatum*, *Mucor racemosus*, *Phoma cava*, *Torula herbarum* were also recorded.

Watermelons are most frequently brought to Lithuania from Russia or Spain. Due to high amount of saccharides and their composition, traders often call them fruits. Contact with soil largely determines the contamination of watermelons with micromycete propagules. Very frequently watermelons are damaged by *Alternaria* and *Cladosporium* micromycetes, but, due to a particular chemical composition, they are also easily infected by *Geotrichum fermentans*, *Rhizopus oryzae,* which participate in fermentation processes. During the period of investigation, the above-mentioned fungi were particularly abundant in watermelons imported from Russia. On the rind of watermelons imported from Spain, black patches were visible and *Alternaria cucumerina*, *Cladosporium cucumerinum* were isolated from them. *Aspergillus ochraceus*, *Geotrichum fermentans, Penicillium digitatum* were isolated from the liquid leaking from a watermelon, while *Acremonium charticola*, *A. pinkertoniae*, *A. strictum* were found in the flesh of injured watermelons. The latter fungi are able to assimilate various substrates. *Pythium aphanidermatum* and *P. ultimum* fungi can be regarded as highly specific. For the first time in Lithuania, *Rhinocladiella spinifera* (= *Phialophora spinifera* = *Exophiala spinifera*) micromycetes were isolated from imported watermelons; these fungi

are usually isolated from palm tree leaves, fallen fruits, bird nests, rotting straw and other substrates. These fungi are known parasites of human respiratory and other organs (Domsh et al., 1980). Various unidentified yeasts were abundant in watermelons.

Bulbous vegetables (onions, leeks, garlic) are favoured for biologically active substances, phytoncides that suppress the action of microorganisms. However, various micromycetes, especially those of the genus *Penicillium*, are abundand on the surface as well as inside these vegetables. In leeks imported from Poland, *Penicillium granulatum* (60%), *P. claviforme, P. clavigerum* (40%) were recorded. *Sclerotinia sclerotiorum* (about 37%), *Rhizopus stolonifer* (about 13%) fungi were also rather abundant in leeks.

Garlic imported from Egypt was damaged by *Aspergillus niger, Cladosporium cladosporioides,* sometimes by *Fusarium oxysporum* and *Mucor silvaticus* fungi.

Chromatographic characteristics on silica gel indicated that in *Fusarium proliferatum* extract four compounds were detected, forming a dark purple fluorescence in the presence of UV light (Fig. 1). The compound with Rf 0.40 in the chloroform–methanol system of solvents (98:2) was identified as the T-2 toxin on comparison with a standard.

Fungi of the genus *Penicillium* prevailed also on onions grown locally and imported from Poland, Holland and other countries (*Penicillium spinulosum*, *P. claviforme, P. italicum, P. expansum*, *P. funiculosum*, *P. granulatum*, etc., detection frequency from 7 to 21%). On onions imported from Holland, more frequent were *Penicillium fuscum*, *P. verruculosum, P. italicum,* sometimes no other fungi were isolated (Fig. 2). The onions imported from Poland were infected with *Penicillium aurantioviolaceum*, *P. expansum, P. fuscum*, *P. granulatum, P. italicum*, *P. lanosum*, *P. lanosocoeruleum*, *P. olivinoviride*. Here *Geotrichum candidum* and *Spegazzinia tessarthra* could be ascribed to prevailing ones. The abilities of the above-mentioned fungi, contaminating vegetables, to synthesize toxic secondary metabolites are not equally studied, but many of them are known as potential mycotoxin producers, therefore, the infected vegetables not just loose marketable appearance, but become hazardous to human health.

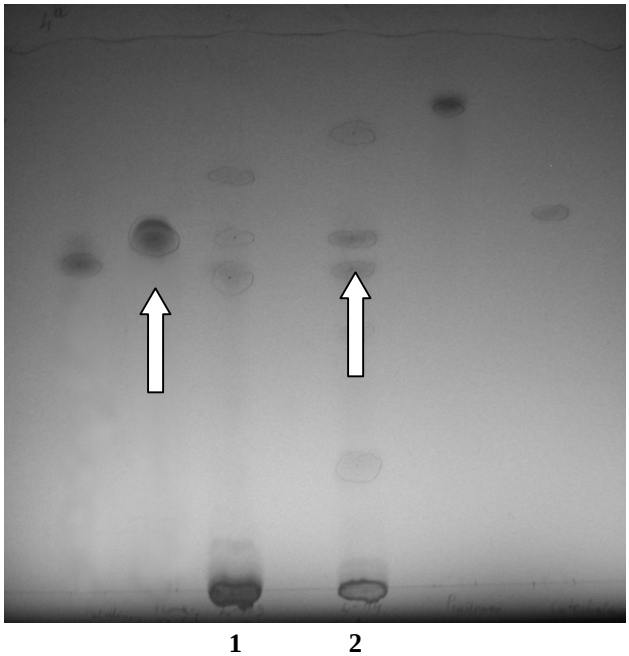

Fig 1. Chromatogram of mycotoxins: 1 – *Fusarium proliferatum* and 2 – *Penicillium verrucosum* (⇧ – patulin)

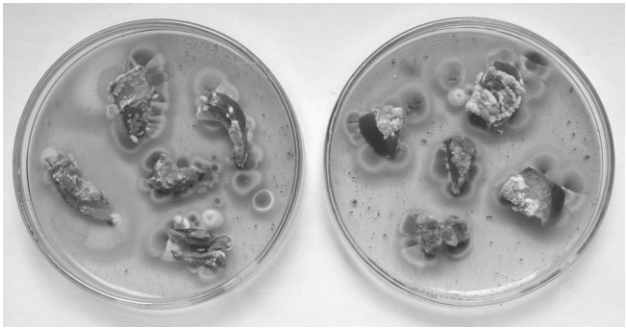

Fig. 2. Micromycetes isolated from onions (Holland)

While vegetables used for food are richer in polysaccharides, fruits and berries include more mono- and disaccharides. In order to enrich the nutrition of people, a lot of berries, grapes, drupes, pomes, citruses and their products are presently marketed in Lithuania. Together with these valuable products micromycetes are also brought in. The products are sometimes contaminated while transporting, storing or selling, because they make a good substrate for various micromycetes and other microorganisms. Meanwhile the quality of raw materials or products considerably worsens because of the activity of contaminating micromycetes. It is emphasized in many literature sources cited in Introduction.

Data on micromycetes brought together with fruits from the regions of subtropical and tropical climate is presented in Fig. 3.

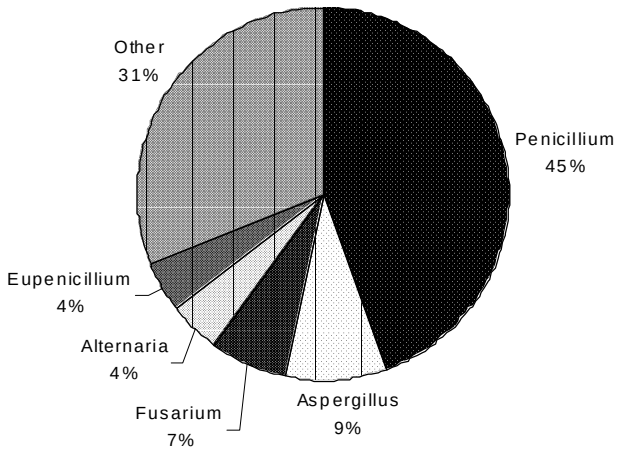

Fig. 3. Distribution of micromycetes genera infecting the imported fruits

Micromycetes of the genus *Penicillium* make the majority (45%) of micromycetes on these fruits. Here micromycetes of the *Eupenicillium* genus (4%) can also be mentioned, as well as fungi of the *Aspergillus* (9%), *Fusarium* (7%), *Alternaria* (4%) genera. About 31% of the isolated micromycetes were ascribed to other genera. The data on micromycete species diversity on the imported drupes and pomes is presented in Fig. 4. Beside fungi of the genus *Penicillium*, which made 31%, also *Aspergillus, Alternaria, Aureobasidium, Cladosporium, Geotrichum* fungi made 6% each. The majority of fungal propagules recorded on raw material of drupes and pomes were ascribed to other, sometimes rather peculiar, genera. Data about the micromycete species recorded on the imported fruits (Table 1) show that some micromycetes are potential toxin producers. We have no data concerning the physiological peculiarities of many micromycetes, although they are frequent on certain imported, stored, marketed and consumed fruits.

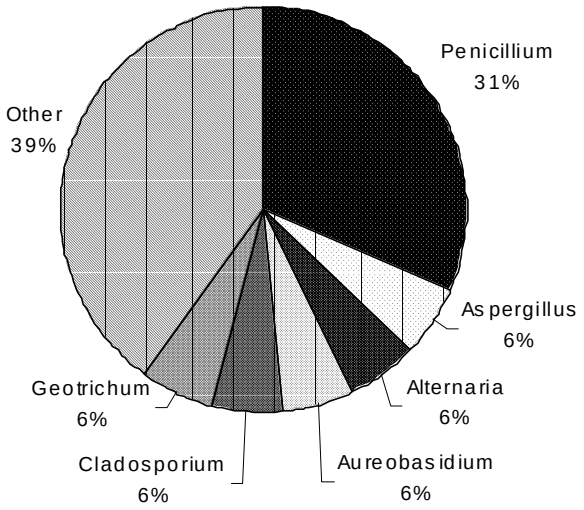

Fig. 4. Distribution of micromycetes genera infecting the imported drupes and pomes

The data presented in Table 1 show that ecological alterations of micromycetes on fruits and other food products can seriously affect their quality, because many micromycetes growing on a favourable substrate, as fruits certainly are, can intensively produce toxic substances. It well agrees with the data presented in Tables 1 and 3, which list potential producers of the identified mycotoxins. Note should be taken of an increased amount of aflatoxin determined in unshelled sunflower seeds imported from Ukraine (32.5 μg kg^{-1}) and its traces in pistachios from Iran (1.7) (Fig. 5, Table 2), poppy seeds from Czech Republic (0.2), and unshelled cedar nuts. A notably high amount of deoxinivalenol was determined in coarse corn flakes imported from Russia, although *Fusarium* fungi, clear producers of this mycotoxin, were not recorded. Probably they had perished in the course of thermal treatment. In unshelled and shelled sunflower seeds zearalenon was detected (15 and 22 μg kg^{-1}, respectively). This mycotoxin was also detected in shelled pumpkin seeds (10 μg kg^{-1}) imported from China and poppy seeds from Czech Republic (13 μg kg^{-1}). Ochratoxins were revealed in a nut and raisin mixture (5.2 μg kg^{-1}), while in seedless raisins and dried cherries patulin was recorded (4–3 μg kg^{-1}, respectively).

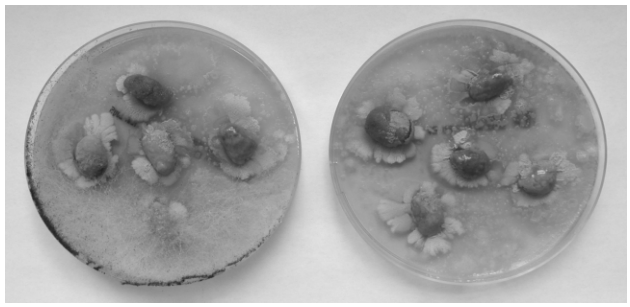

Fig. 5. Micromycetes isolated from pistachios (Iran)

The group of drupes, including rosaceous plants (cherries, sweet cherries, plums, peaches, apricots, etc.) with the addition of the pomes (apples and pears), was most frequently infected with *Alternaria alternata, Ulocladium chartarum, Penicillium digitatum, P. expansum, P. granulatum, P. italicum*. In sweet cherries imported from Hungary, *Ulocladium chartarum, Verticicladium trifidum* (detection frequency reaching 68%) dominated, less frequent being *Aspergilus niger* and *Penicillium italicum*. The latter micromycetes, however, are known as producers of toxic secondary metabolites (Lugauskas, 2005), therefore, their development in sweet cherries causes not just great economic losses, is ecologically unfavourable, but also presents a real hazard of toxicoses. Meanwhile in sweet cherries from Poland micromycetes characterized by active production of hydrolytic enzymes dominated. *Aureobasidium pullulans, Penicillium biforme, P. oxalicum, P. restrictum, Fusarium proliferatum* micromycetes isolated from cherries are also able to synthesise mycotoxins. *Alternaria alternata, Ulocladium chartarum, Acremonium roseolum, Penicillium expansum* fungi comprised 67% of mycological contamination determined on plums imported from Hungary. Some of them are characterized by a various degree of pathogenicity and ability to synthesize toxic secondary metabolites.

From apricots imported from Spain, only *Paecilomyces javanicus* micromycetes were isolated. We have no data concerning the physiological peculiarities of these fungi, because they are rare in Lithuania. Probably during their growth or gathering, the apricots were treated with fungicides. On the skin of apricots imported from France black round spots were visible; *Cladosporium tenuissimum* fungi were constantly isolated from them; later they covered the whole fruit surface with a dark coating. On apricots from Hungary, *Cladosporium cladosporioides* and *Penicillium italicum* micromycetes were most abundant. The study results have shown that apricots grown in various countries are

Table 1. **Micromycete species isolated from imported berries, fruits, nuts and other products**

Product	Country	Micromycetes species
Strawberry	Poland	*Mucor ramannianus, Penicillium biforme, P. granulatum*
Grape	Chile	*Aspergillus niger, Eupenicillium brefeldianum, Exophiala mansonii, Penicillium corymbiferum, P. paxilli*
	Italy	*Geotrichum fermentans*, various species of yeast
	Hungary	*Geotrichum fermentans, Penicillium canescens, P. cyclopium, P. frequentans, P. palitans*, various species of yeast
Sweet cherry	Hungary	*Alternaria* spp., *Aspergillus niger, Botrytis cinerea, Penicillium italicum, Ulocladium chartarum, Verticicladium trifidum*
	Poland	*Absidia cylindrospora, Dipodascus armillariae, Geotrichum candidum, Fusarium lateritium, Penicillium granulatum*
Cherry	Poland	*Aspergillus niger, Aureobasidium pullulans, Ctenomyces serratus, Fusarium proliferatum, Geotrichum fermentans, Hormonema prunorum, Penicillium biforme, P. oxalicum, P. restrictum, Sclerotinia sclerotiorum*
Plum	Hungary	*Acremonium roseolum, Alternaria alternata, Aureobasidium prunicola, Penicillium expansum, P. italicum, Pleospora infectoria, Ulocladium chartarum*
Apricot	Spain	*Paecilomyces javanicus*
	France	*Cladosporium tenuissimum, Fusarium solani, Penicillium corylophilum, P. damascenum, Ulocladium chartarum*
	Hungary	*Cladosporium cladosporioides, Fusarium solani Penicillium italicum*
Peach	Spain	*Botrytis cinerea, Cunninghamella elegans, Penicillium granulatum, P. italicum, Rhizopus stolonifer*
	Greece	*Ascochyta pruni, Aspergillus candidus, Aureobasidium prunicola, Eurotium niveoglaucum*
Banana	Ecuador	*Acremonium charticola, Fusarium moniliforme, F. sporotrichioides, Nectria haematococca*
Orange	Spain	*Botrytis cinerea, Eupenicillium brefeldianum, Penicillium aurantioviolaceum, P. corymbiferum, P. digitatum, P. expansum, P. islandicum, P. italicum, P. variabile*
	Morocco	*Cunninghamella vesiculosa, Geotrichum fermentans, Penicillium expansum, Thamnidium fulvum*
Tangerine	Morocco	*Eupenicillium brefeldianum, Leptodontium boreale, Penicillium chrysogenum, P. italicum, P. janthinellum, P. verruculosum*
	Spain	*Alternaria citri, Penicillium atramentosum, P. islandicum, P. italicum, P. verruculosum*
Grapefruit	Africa	*Penicillium chrysogenum, P. digitatum,, P. nalgiovense, Scopulariopsis acremonium*
	Israel	*Aspergillus niger, Fusarium oxysporum, Penicillium duponti, P. piceum, P. purpurogenum, P. wortmanni, Stemphylium atrum*
Lemon	Argentina	*Aspergillus penicilloides, Cladosporium macrocarpum, Gliocladium virens, Penicillium daleae, P. digitatum, P. expansum, P. verrucosum*
	Turkey	*Aspergillus athecius, Eupenicillium brefeldianum, Penicillium chrysogenum*
Avocado	South Africa	*Alternaria alternata, Aspergillus fumigatus, A. niger, Mortierella polycephala, Penicillium chrysogenum, P. granulatum, P. purpurogenum, P. steckii, Verticillium psalliotae*
Apple	Poland	*Aureobasidium pullulans, Mucor ramannianus, Penicillium chrysogenum, P. expansum, P. digitatum, P. granulatum, P. italicum, P. lanosoviride, P. melinii, P. viridicatum, Trichoderma harzianum*
Pear	Poland	*Aspergillus niger, Geotrichum fermentans, Penicillium italicum, P. nalgiovense*, various species of yeast
Desiccated cherries	China	*Aspergillus duricaulis, A. niger, Penicillium aurantiogriseum, P. expansum, Trichothecium roseum, Mycelia sterilia*
Desiccated strawberries	China	*Aspergillus clavatus, A. duricaulis, A. fumigatus, A. niger, A. ochraceus, A. oryzae, A. penicillioides, Eupenicillium brefeldianum, Penicillium atramentosum, P. aurantiogriseum, P. brevicompactum, P. expansum, P. italicum, P. stoloniferum, Mycelia sterilia*
Desiccated mango	Thailand	*Aspergillus duricaulis, A. fumigatus, A. oryzae, Cladosporium cladosporioides, Penicillium citreoviride, P. expansum, P. italicum, P. verrucosum, Mycelia sterilia*

Table 1 (continued)

Cashew nuts	India	*Penicillium aurantiogriseum, P. corymbiferum, P. expansum, Rhizopus oryzae, Rh. stolonifer,Mycelia sterilia*
In-shell cedar nuts	Ukraine	*Aspergillus flavus, A. fumigatus, A. niger, Penicillium expansum, P. granulatum, Penicillium spp., P.verruculosum, P. viridicatum, Mycelia sterilia*
Almonds	America	*Aspergillus niger, Penicillium brevicompactum, P. verrucosum, Rhizopus oryzae, Mycelia sterilia*
Pistachios	Iran	*Aspergillus fischeri, A. fumigatus, A. niger, A. ochraceus, A. pulverulentus, Penicillium expansum, P. verrucosum, Mycelia sterilia*
Nuts and raisins mix		*Aspergillus granulosus, A. niger, A. sydowii, Aureobasidium pullulans, Rhizopus oryzae, Mycelia sterilia*
Shelled squash seeds	China	*Aspergillus niger, A. ustus, Rhizopus oryzae, Mycelia sterilia*
Sunflower seeds in shell	Hungary	*Aspergillus clavatus, A. fischeri, A. fumigatus, A. niger, A. ustus, Myceliophthora vellerea, Mycelia sterilia*
Poppy seeds	Czechia	*Alternaria alternata, A. cinerariae, Aspergillus fumigatus, Cladosporium cladosporioides, Mortierella humilis, Penicillium viride, Rhizomucor pusillus, Rhizopus oryzae, Mycelia sterilia*
Seedless raisins	Iran	*Alternaria alternata, Aspergillus niger, Mycelia sterilia*

Table 2. **Mycotoxins in imported nuts, dry fruits and other products**

Product	**Country**	**Amount of mycotoxins, μg kg^{-1}**					
		Aflatoxins	**Deoxynivalenol**	**T-2 toxin**	**Zearalenone**	**Ochratoxins**	**Patulin**
Cashew nuts	India	0	-	-	-	-	-
In-shell cedar nuts	Ukraine	0.1	-	-	-	-	-
Peanuts without seed-coating	Greece	0	-	-	-	-	-
In-shell walnuts	Ukraine	0	-	-	-	-	-
Almonds	America	0	-	-	-	-	-
Pistachios	Iran	1.7	-	-	-	-	-
Nuts and raisins mix		0	-	-	-	5.2	0
Shelled squash seeds	China	0	0	0	10.0	0.3	-
Sunflower seeds in shell	Ukraine	32.5	0	0.4	15.0	0	-
Shelled sunflower seeds	Hungary	0	0	0	22.0	0	-
Poppy seeds	Czechia	0.2	0	0	13.0	0	-
Seedless raisins	Iran	-	-	-	-	2.9	4.0
Desiccated cherries	China	-	-	-	-	1.9	3.0
Desiccated strawberries	China	-	-	-	-	0.7	0
Desiccated mango	Thailand	-	-	-	-	1.3	0
Large oatflakes	Russia	0	150.0	14.3	0	0	-

Table 3. **Micromycetes isolated from cocoa beans and their products**

Product	**Micromycetes species**
Cocoa beans	*Alternaria alternata, Aspergillus flavus, A. repens, A. ustus, A. niger, A. niveus, A. pseudoglaucus, Mucor hiemalis, M. circinelloides, Mycelia sterilia*
Ground cocoa	*Aspergillus flavus, A. sulphureus, A. terreus, A. sydowii, Cladosporium herbarum, Penicillium carneum, P. expansum, P. corymbiferum, Monilia* spp.*, Mycelia sterilia*
Cocoa squeeze	*Aspergillus sydowii, Penicillium lividum, P. commune, P. janthinellum*
Cacao butter	*Alternaria* spp.*, Aspergillus ochraceus, A. ruber, Mycelia sterilia*
Cacao butter with powder admixture	*Aspergillus flavus, A. oryzae, Fusarium proliferatum, Mucor circinelloides, Mycelia sterilia*
Chocolate	*Aspergillus fischeri, A. niger, A. wentii, Dipodascus* spp.

Table 4. **Mycotoxins in cocoa products**

Product	Amount of mycotoxins, mg kg^{-1}				
	Aflatoxins	Deoxynivalenol	T-2 toxin	Zearalenone	Ochratoxins
Cocoa beans from a bin	0.018	0.300	0.016	0.550	0.031
Crushed cacao beans after roasting and shelling	0.020	0.230	0.043	0.600	0.066
Grounded cacao after roasting	0.019	0.130	0.035	0.650	0.017
Grounded cacao after debacterisation	0.027	0.125	0.037	0.700	0.018
Cacao squeeze (after press)	0.050	0.225	0.052	0.800	0.065
Cacao butter (after press)	0.005	0	0.001	0.070	0
Cacao powder from squeeze	0.040	0.048	0.068	0.850	0.020
Chocolate „S“	0.008	0.050	0.004	0.068	0.0002
Cacao butter (further added into chocolate)	0.006	0.30	0	0.150	0
Cacao squeeze (left after butter production, ground and added into chocolate	0.030	0.180	0.057	1.0	0.025
Ground cacao after heating (debacterisation)	0.040	0.430	0.038	0.50	0.028
Cacao squeeze	0.037	0.050	0.050	0.70	0.031
Cacao butter with powder admixture	0.013	0.430	0	0.20	0.005

infected with micromycetes of different species ascribed to the same genera. Their toxicity potential can hardly be defined. On peaches brought from Spain, *Botrytis cinerea, Cunninghamella elegans, Rhizopus stolonifer* and *Penicillium granulatum* dominated at the moment of the study (detection frequency up to 67%) together with *Penicillium italicum* fungi which were detected less frequently. The above-mentioned fungi easily assimilate various saccharides, other carbon sources and synthesize complex, frequently toxic secondary metabolites (Cole, Schweikert, 2003).

In bananas imported from Equador, fungi synthesising and excreting toxic substances of trichotecenes group (*Fusarium moniliforme, F. sporotrichioides*) were recorded; their detection frequency reached 50%. The fungi were accompanied by *Nectria haematococca, Acremonium charticola* micromycetes. It should be noted that the mentioned fungi can develop under conditions of a low temperature which is usually maintained in storehouses. It has been determined that *Fusarium moniliforme* fungi are tolerant to an increased concentration of NaCl in the medium and are able to develop under conditions of oxygen deficiency or even in anaerobic conditions.

Oranges imported from Spain were mostly damaged by *Botrytis cinerea* micromycetes. It is difficult to define their origin as these are cosmopolitan fungi, developing under diverse conditions on a wide variety of substrates. *Eupenicillium brefeldianum* fungi were intensively developing on oranges; their detection frequency reached 66.7%. These fungi synthesise and excrete fulvic acid and other metabolites (Samson and Frisvad, 2004). *Penicillium italicum*, *P. variabile, P. chrysogenum*, *P. janthinellum*, *P. verruculosum* were recorded in oranges. Under certain conditions, all these micromycetes are able to produce toxic secondary metabolites; therefore their development on oranges is highly unacceptable and hazardous to human health.

On mandarins imported from Morocco and Spain, *Leptodontium boreale* fungi were detected; the data on their physiological peculiarities and excreted metabolites is scarce. On Spanish mandarins, *Alternaria citri* fungi were recorded. Usually the spores of this fungus get into a plant during its blooming, and later the formed fruit starts rotting from the inside. These fungi synthesise altenusin which is ascribed to the group of altenuenes and related metabolites (Cole and Schweikert, 2003). Grapefruits imported from Africa are frequently damaged by micromycetes of the genus *Penicillium*; *P. chrysogenum*, *P. digitatum, P. nalgiovense. Scopulariopsis acremonium* micromycetes were also present. Grapefruits imported from Israel were infected with *Penicillium dupontii*, *P. purpurogenum*, *P. piceum, P. wortmannii* and *Aspergillus niger* micromycetes. Yellow grapefruits from Israel are frequently damaged by *Fusarium oxysporum,* sometimes by *Stemphylium atrum* (= *Ulocladium atrum*) fungi which is a highly potential mycotoxin producer.

Lemons brought from Argentina were frequently contaminated by *Penicillium daleae*, *P. digitatum* micromycetes; their detection frequency reached 43%. *Cladosporium macrocarpum*, *Gliocladium virens* (29%) were also recorded. Toxins excreted by these fungi can be toxic to plants and animals (Domsch et al., 1980; Cole, Schweikert, 2003). From lemons imported from Turkey, *Eupenicillium brefeldianum*, *Aspergillus ochraceus* and *Penicillium chrysogenum* fungi were isolated; under certain conditions they can become active producers of

mycotoxins. On citrus fruits, *Eupenicillium brefeldianum*, *Penicillium italicum, P. digitatum, Aspergillus niger* and other fungi synthesizing toxic secondary metabolites were recorded.

The chemical composition of avocados, grown in the tropical climate, strongly differs from that of citruses and drupes; it resembles vegetables. By the abundance of oils they almost equal nuts. So micromycetes of other species were also recorded on them. *Alternaria alternata*, *Aspergillus fumigatus*, *Mortierella polycephala* micromycetes prevailed; their detection frequency reached about 68%. The detection frequency of *Verticillium psalliotae*, *Aspergillus niger, Penicillium chrysogenum, P. granulatum*, *P. purpurogenum*, *P. steckii* exceeded 30%.

Analysis of microycetes detected on apples from Poland revealed the prevalence of *Penicillium* fungi (*P. expansum* (75%), *P. lanosoviride*, *P. chrysogenum, P. italicum, P. viridicatum*); other species were less frequent. These fungi are known as producers of toxic secondary metabolites. *Aureobasidium pullulans*, *Trichoderma harzianum*, *Mucor* fungi were isolated from apples. The role of the latter fungi in the processes of fruit destruction is insufficiently studied. *Aspergillus niger, Penicillium italicum*, *P. nalgiovense*, *Geotrichum fermentans* and yests of various species dominated on pears imported from Poland. In short-term storage premises, *Penicillium italicum* micromycetes were frequent; they could have settled on apples from the environment, primarily from the citruses.

A specific composition of micromycete species was determined on seeds of various plants or foodstuffs produced of them. For example, on soybean meal from Holland, yeast-like fungi of the *Candida albicans*, *Rhodotorula rubra*, *Saccharomyces cerevisiae* species prevailed; on rapeseed meal from Belarus, *Rhizomucor pusillus*, *Rhizopus oligosporus*, *Rhodotorula rubra, Saccharomyces cerevisiae,* and on corm imported from Hungary, *Fusarium culmorum*, *F. poae, F. sambucinum*, *F. graminearum* were frequent. *Rhodotorula rubra, Candida albicans* were also present. Sterile mycelium (*Mycelia sterilia*) comprised a rather high amount of propagules in all samples. *Fusarium poae* fungi prevailed on sunflower seeds from Ukraine.

The mycotoxins aflatoxins, deoxinivalenol, T-2 toxin, zearalenone and ochratoxin were detected in cocoa beans imported from Central America; here they are used in the production of certain confectionery products (Table 4). It should be noted that not all producers of the above-mentioned mycotoxins survive after the thermal treatment of beans. Meanwhile the toxins produced by them remain inactive in the beans. It is mostly true regarding active producers of deoxinivalenol, T-2 toxin, zearalenone from the genus *Fusarium*. In many cases they could be isolated neither from cocoa beans nor from their products. Only from cocoa butter with powder admixture *Fusarium proliferatum* fungi were isolated; they are able to excrete fumonisin AK_1, fusaproliferin, proliferin, moniliformin, i.e. compounds characterized by toxicity. Meanwhile on beans and in their products, micromycetes of the genera *Aspergillus, Penicillium, Alternaria* dominated (Table 3).

Tests on the acute toxicity of micromycetes that have intensively excreted pigments into media were performed with 12 week old BALB/c mice of both sexes. The mice were kept according to European Convention for the Protection of Vertebrate Animals for Experimental and other Scientific Purposes (EU Commission Directive 92/69/EEC).

The BALB/c mice did not react to the a single dose of a freshly prepared mixed aquatic suspension of four fungi isolated from cocoa beans, which was administered *per os*.

CONCLUSIONS

1. Each shipment of food products from abroad is accompanied by microorganisms of various species; their ecological, economic and sanitary significance can hardly be defined in advance.

2. Microorganisms detected in food products imported from various countries get on them from different sources such as growing localities, transporting vehicles, selling environment and measures, people involved.

3. Nowadays plenty of raw materials intended for food and specifically treated products of diverse origin and composition are imported. The degree of their contamination with microorganisms differs. A certain part of the imported goods are treated applying chemical, physical and biological measures. Therefore, the mycological condition of the imported raw material and products is uneven.

4. Micromycetes of the genera *Aspergillus, Penicillium, Mucor, Rhizomucor, Rhizopus, Alternaria, Cladosporium, Acremonium, Verticillium, Fusarium* are most abundantly brought into the country with food raw materials and products. A frequent and important feature, typical of the majority of the mentioned species and their strains, is their ability to synthesize and excrete toxic metabolites – mycotoxins. Their chemical composition and impact upon the environment are rather wide and ambiguous.

5. Some micromycetes imported to Lithuania together with food products are rarely encountered in the country; therefore, it is impossible to define their impact upon the food quality without additional research. Other micromycetes are cosmopolitans, able to assimilate various substrates and produce the already known toxic secondary metabolites hazardous to human health. When they get in the environment, they can become hazardous pollutors of the environment and food products.

6. Micromycetes imported with raw materials, especially those able to develop and function at higher temperatures, can spoil the established course of food processing technologies and deprive of the possibility to

obtain food products of the required quality. Therefore a constant microbiological control of the quality of food products is essential, especially regarding the accumulation of mycotoxines.

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MIKROMICETŲ, ATVEŽTŲ IŠ UŽSIENIO SU ĮVAIRIAIS AUGALINĖS KILMĖS MAISTO PRODUKTAIS, EKOLOGINĖ IR SANITARINĖ REIKŠMĖ

S a n t r a u k a

2003–2006 m. ištyrus mikromicetus, aptiktus ant įvairaus pavidalo augalinės kilmės maisto žaliavos ir produktų, atvežtų ir

realizuojamų Lietuvoje, nustatyta jų rūšių sudėtis, išaiškintos galimybės sintetinti bei išskirti į aplinką toksiškus antrinius metabolitus ir plisti aplinkoje. Nustatyta, kad daug su augalinės kilmės maisto žaliava ir produktais atvežtų mikromicetų geba prisitaikyti prie naujų ekologinių sąlygų, patekti į prekybines, gamybines ir gyvenamąsias patalpas ir praturtinti vietinėmis sąlygomis esančių mikromicetų rūšių įvairovę, praplėsti funkcines, ne visada žmonėms naudingas, destrukcines galimybes ir ne tik tapti ekonominių nuostolių priežastimi, bet ir sukelti pavojų žmonių sveikatai. Kai kurių rūšių mikromicetai yra kvėpavimo organų ligų, astmos, įvairių alerginių negalavimų, odos ligų priežastis, neretai sukelia įvairių organų gilumines mikozes, o jų išskiriami toksiški antriniai metabolitai ženkliai pablogina maisto kokybę, kelia nuolatinį pavojų jo saugai. Remiantis atliktų tyrimų duomenimis, minėti klausimai aptariami šiame straipsnyje.

Raktažodžiai: grybai, tarša, toksinai, daržovės, vaisiai, grūdai, įvežti produktai