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TOXIC MICROMYCETES IN GRAIN RAW MATERIAL DURING ITS PROCESSING

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Abstract: In 2003–2005 micromycetes were isolated and identified from wheat, barley, rye, buckwheat grain brought into mills or from processing enterprises. Contamination of the produced flour with micromycete propagules (cfu g^{-1}), changes in micromycete diversity and abundance in the course of flour storage, preparation and baking of bread, production of groats or other food products and fodder were determined. Most attention was given to widely distributed micromycetes, known producers of toxins: *Alternaria alternata*, *Aspergillus candidus*, *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A.* (=*Eurotium*) *repens*, *Fusarium culmorum*, *F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. oxysporum*, *F. poae*, *F. sporotrichioides*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. cyclopium*, *P. daleae*, *P. expansum*, *P. funiculosum*, *P. roqueforti*, *P. urticae*, *P. verruculosum*, *P. viridicatum*, *Phoma exiqua*, *Rhizomucor pusillus*, *Rhizopus stolonifer*, *Trichothecium roseum*. Abilities of these micromycetes to produce secondary toxic metabolites were determined as well as possible hazard caused to people consuming the contaminated products.

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INTRODUCTION

The data of the Food and Agriculture Organization (FAO) [21] reveal that about 25% of foodstuffs produced worldwide are contaminated with mycotoxins. Constant consumption of the food contaminated with mycotoxins exerts serious risk of disorders of the respiratory, digestion, nervous, and other organism systems. Effects of ecological impact of mycotoxins should also be considered [5, 6, 9, 10, 11, 22, 49].

Presently human nutrition contains plenty of products of plant origin produced from grain and seeds of cereals and pulses. Microbiological analyses of the grain enabled determination of the diversity and abundance of microorganisms inside and on the surface of each grain and to ascertain potential hazard of contamination with toxic secondary metabolites [8, 15, 20, 24, 31, 36, 64, 73, 89]. Quality of food raw material and foodstuffs is usually evaluated based on physical, chemical, and microbiological criteria. One of the main factors influencing the quality of food raw material and foodstuffs is their contamination with microorganisms, and its level in the product. In food-stuffs produced from cereal grain the process of microbial contamination starts with the beginning of grain and seed ripening, continues through grain harvesting, storage, processing, selling and ends only after consumption of the ready foodstuff. During all stages of their development plants as well as yielded grain and seeds are in close contact with live and non-living objects of the surroundings and the superabundance of microorganisms existing in the environment. Micromycetes are characterised by very specific biodiversity, physiological, and adaptational properties; they easily contaminate materials of plant origin and use them to satisfy their requirements [1, 7, 15, 24, 29, 43, 47, 54, 72]. Presently, scientists of many fields indicate the hazard

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caused by toxic secondary metabolites synthesized and excreted by micromycetes; these metabolites can cause functional, respiratory, allergic, carcinogenic, psychical disorders of human health [23, 28, 30, 31, 32, 35, 36, 50]. Each micromycete species is characterized by specific metabolites; the intensity of their synthesis depends upon cultural features of an individual and environment where toxin synthesis takes place, primarily upon the substrate. The origin of microorganisms detected on grain is different. Microorganism propagules get on grain in different ways, most often with dust from soil [48], from the surface of plant remnants [46], during harvesting, transportation, storage, and processing. The level of microbial contamination of grain depends upon the abundance of propagules, species diversity of occasionally formed communities, and possibilities for their mutual functional activities. Climatic conditions of Lithuania are favourable for the development and spreading of micromycetes of the *Penicillium* Link, *Fusarium* Link, *Aspergillus* Link, *Mucor* Fresen., *Rhizopus* Ehrenb., *Rhizomucor* Lucet & Constantin, *Aureobasidium* Viala & G. Boyer, *Cladosporium* Link, *Drechslera* S. Ito, *Sclerotinia* Fuckel, *Verticillium* Nees genera. Many micromycetes of the above-mentioned genera are able to synthesize toxins that are hazardous to people and animals, are rather stable, and resistant to temperatures. They accumulate in grain and considerably alter the grain quality which become poor in nutritions, bitterly, and even hazardous to human health [38, 40, 41, 53, 55, 58, 62, 63].

While processing grain into flour or groats, micromycete propagules do not just get into the ready products, but spread widely in the environment, contact new substrates, settle in an unusual environment where they are influenced by new factors. Under such conditions functional characteristics of micromycetes and, consequently, metabolites change [39, 42, 59, 66, 67, 69].

Some fungi are characterized by a possibility to excrete volatile substances, which are associated with toxicity of these fungi. The excreted toxins easily vaporize and diffuse in the surrounding; they come into contact with the foodstuffs and intermix with their components [71, 73, 89]. Results of long-term investigations concerning alkaloids synthesized by fungi of the *Penicillium* genus and their toxic properties have been presented by Russian scientists [78–83, 94, 95]. The data on fungi of the *Aspergillus* and *Penicillium* genera able to synthesize toxins containing nitrogen compounds is also available [84].

During processing, microorganisms present on the surface of grain and plant seeds get into flour, groats, and other products. The amount of microorganisms in flour and groats depends upon the amount of seed-coat in them. It has been determined [14] that in the superfine flour the amount of micromycetes is from 9 to 14%, in first-rate flour – from 11 to 32%, and in second-rate flour – from 21 to 56% compared with the number of micromycetes recorded on whole grain.

In the course of dry and moist grain cleaning, as well as during grain sorting according to density, no changes in the amount of toxins were revealed [97]. Meanwhile when the contaminated grain is milled, a large part of fungal propagules together with the produced toxins get into the ready products [91]. There is some evidence [56, 57] that in flour the amount of mycotoxins excreted by micromycetes of the *Fusarium* genus is considerably reduced when the grain surface is dry cleaned during the process of flour production. However, an increase in the amount of toxins in bran is revealed. Such a reduction in the amount of toxins can be explained by the impact of a high temperature during the grain milling. However, ochrotoxin A synthesized by *Penicillium* fungi and some other mycotoxins are rather resistant to high temperatures. Some specialists try to explain the reduction of the toxins in certain fractions of flour by an uneven distribution of toxins in whole grain [97].

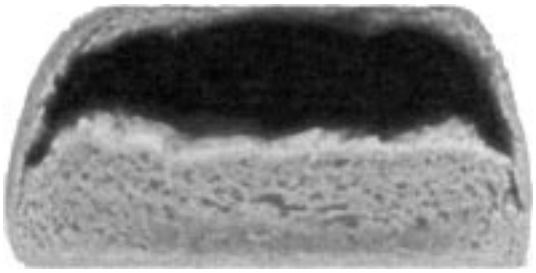

Figure 1. Wheat bread infected with bacteria Bacillus subtilis ("potato disease").

Micromycetes of the *Aspergillus*, *Mucor*, *Penicillium* and other genera most often cause bread mouldering. The impact of microbiological contamination of flour upon mouldering of bread is explained by the process when bacterial exoenzymes destroy the microstructure of the bread pulp and the amount of water moving from the pulp towards the crust increases which consequently induces growth and development of micromycetes [75]. It has been determined that such bread can contain substances hazardous to human health [3, 75, 76]. Besides the above-mentioned factors influencing the lasting of bread, bacteria *Bacillus subtilis* (Ehrenb.) Cohn play an important role because they cause "potato disease" of bread. It has been determined that vegetative cells of *Bacillus subtilis* do not survive at a temperature of 75–80ºC, while spores remain viable at 120ºC for 1 hour. Therefore, these bacteria are often recorded in bread. Optimal conditions for their spread and growth are: temperature 40ºC, pH 5–10 [74]. Bacteria and fungi often function simultaneously, thus increasing the chances of production losses together with sanitary hazards (Fig. 1).

The aim of the present research was to investigate the abundance, diversity, and alterations of potential toxin producing micromycetes in cereal grain during different stages of raw material processing, to reveal factors determining the intensity of toxin accumulation and possibilities of their survival in raw material as well as in ready products, and to evaluate the level of mycotoxin contamination of the ready products and the risk imposed on human health.

MATERIALS AND METHODS

Investigated premises. In 2003–2005, the contamina–tion of barley, wheat, rye, buckwheat, and other grain with micromycete propagules (cfu g^{-1}) during different technological processes was investigated

Mycological conditions of one bakery and its mill as well as of the used raw material were evaluated. The building of the bakery is old, its brick walls are in some places cracked and crumbled. In the production premises sanitary and hygienic requirements are strictly followed, but some specific conditions - relative humidity in the premises reaching 80–90%, temperature 25–30ºC, in–sufficient ventilation - are favourable for the development of micromycetes. In the investigated bakery the bread was produced from the ingredients presented in Table 1.

Technological process of bread production includes storage of raw materials, dough fermentation, dough processing, bread baking, bread cooling and storing. In the mill of the bakery the contamination of cereal grain before milling and the flour produced from it was evaluated.

In order to reveal the efficiency of the extrusion upon the abundance of micromycete propagules in grain, tests were performed. As test objects by-products of the groat production unit (bran, small grain, fodder flour) were chosen. In the extruder crushed grain of flour are exposed to a pressure of 1.4–1.5 MPa for 30 seconds, due to the friction the products reach the temperature of 180ºC and form a homogeneous mass. On leaving the extruder, as the pressure changes, the whole mass bulges and “bursts”. After 30 seconds in the extruder the amount of humidity in the product reduces by 50%. Under the impact of pressure and temperature, the activity of the majority of micromycetes reduces and some of them are eliminated.

The technological process of food raw material and foodstuffs production was divided into the following main technological stages: cleaning grain of impurities, hydrothermal treatment, grain sorting, shelling, sorting of the acquired products, and kernel treatment.

Barley, wheat, and buckwheat grain intended for groats production were dried, cleaned from light organic, mineral, and metal impurities, small grain and seeds of other plants removed, grain sorted according to size. Cleaned and sorted grain were shelled in the scrub engine. During this process the grain surface is cleaned of adhering soil, dust, partially and sometimes totally the seed-coat, aleuronic layer, and the germ. Barley groats are produced after such grain treatment.

While producing buckwheat groats, different from barley groats, after the first removal of impurities the following method of buckwheat treatment was used: vaporization, drying and cooling. Buckwheat grain were vaporised for 5 min under pressure of 0.25–0.30 MPa and a temperature of 140ºC. Later, the buckwheat grain was kept for 30 min in a granary so that moisture could spread evenly in the whole mass of grain. The grain was dried in contact vapour driers. Hydrothermal treatment of buck-wheat grain increases the coefficient of grain shelling.

Table 1. Raw materials, additives, and ready products in the bakery.

Raw materials	Additives	Ready production
Wheat flour	Flax-seed	Wheat bread
Scrub wheat flour	Dry plums (Argentina)	Coarse rye bread (packed)
Rye flour	Shelled pumpkin seeds (China)	White wheat bread (sliced)
Salt	Caraway seeds	
Rye malt	Dry calamus	
	Starch syrup	
	Raisins (Iran)	

Grain, flour, and product sub-samples for microbiological analyses were taken directly in the production areas forming one sample of 10 sub-samples of grain, flour, groats, and other products. For analysis, 172 wheat, 148 barley, 62 rye, 26 buckwheat samples taken as grain, flour, groats, bran and other products were used.

Isolation and identification of micromycetes. Samples of grain, seeds, and other food-stuffs of plant origin were analysed following the earlier described methods [2, 52, 61, 65, 73, 77, 87, 96]. The analysis of each sample was made with three replications. Colonies and micromycete isolates, in order to obtain pure cultures, were cultivated for 5–7 days at a temperature of 28 ± 2ºC on standard malt, Czapek and corn extract media. Fungi were ascribed to certain systematic groups following the system proposed in [34].

Systematic position of fungi was determined according to [12, 13, 18, 19, 43, 44, 45, 46, 47, 60, 70, 85, 86, 88, 90, 93, 96].

Evaluation of micromycete toxicity. The selection of fungi able to synthesize secondary metabolites was tested applying cultivation on standard Czapek agar, Czapek yeast extract agar (CYA), and yeast extract - sucrose agar (YES) media for 7–14 days at 28ºC. Strains - potential producers of mycotoxins - showed significant changes in the colour of colonies and abundant excretion of pigment into CYA and YES media [25, 26, 27, 33].

Thin-layer chromatography method was used for the estimation of toxins produced by fungi. Silica gel 60 with fluorescent indicator UV254 (Mackerey-Nagel) was used. Selected fungal strains were grown on malt agar (MA) for 14 days; biomass was collected and extracted with 96º ethanol. CYA and YES media were used additionally for estimation of the influence of substrate on the ability to produce secondary metabolites. 0.05–0.1 ml of extract and standard toxins were analyzed chromatographically. The separation of compounds was carried out in the system of solvents toluene-ethyl acetate-formic acid (5:4:1). Produced mycotoxins were identified according to Rf (distance of compound/distance of solvent) and fluorescence in the UV, as compared with standards [9,

16, 17]. The amount of patulin produced by fungus *Penicillium* spp. under different growing conditions was determined using international standard [37, 92].

Determination of toxins was performed by the ELISA method. Extraction of mycotoxins and tests were performed according to manufacturer's instruction. The VERATOX®, Alotox (total), VERATOX®DOH5/5, VERATOX®-Ochratoxin A, Aflatoxin, T2 toxin, zearalenone, and RIDACHREEN® Ochratoxin A test kits (R-Biopharm AG, Germany) were used for the analysis.

RESULTS AND DISCUSSION

Micromycetes get into the bakery with raw material, mostly with flour made in the bakery mill or brought from other regions of the country. In Lithuania bread is usually baked from wheat and rye flour; the contamination of the flour with micromycetes is uneven and depends upon many environmental factors that affect the grain up to the moment when it turns into flour. During technological processes of milling the abundance and species diversity of micromycetes in grain evidently changes (Tab. 2).

In wheat grain intended for milling the number of micromycete propagules was 5.0×10^3 cfu g^{-1}, after cleaning of grain surface in scrub engines – 4.7×10^3 cfu g^{-1}, after milling in rolling-mill and sorting the contamination of flour made 3.1×10^3 cfu g^{-1}, of fodder flour – 4.8×10^3 cfu g^{-1}, of bran – 3.5×10^3 cfu g^{-1}. It can be concluded, therefore, that in the course of processing, after dry cleaning of grain in scrub engines, the number of micromycete propagules is reduced. The majority of micromycete propagules get into fodder flour and bran.

In the course of flour production, in grain and its products potentially toxic *Alternaria alternata*, *Fusarium culmorum*, *F. sporotrichioides*, *Penicillium expansum*, *P. verrucosum*, etc. micromycetes prevailed.

Table 2. Micromycetes recorded in raw material during various technological stages of flour production.

Sample	Number of propagules cfu g^{-1}	Identified species	Prevailing micromycete species (population density > 40%)
Grain from elevator	5.0×10^3	14	*Alternaria alternata* *Cladosporium herbarum* *Fusarium culmorum* *Fusarium sporotrichioides*
Grain before first treatment with scrub engine	4.2×10^3	16	*Acremonium murorum* *Aspergillus niger* *Alternaria alternata* *Fusarium culmorum* *Fusarium sporotrichioides*
Grain after first treatment with scrub engine	4.2×10^3	16	*Acremonium murorum* *Alternaria alternata* *Aspergillus niger* *Fusarium culmorum* *Fusarium sporotrichioides* *Penicillium chrysogenum*
Grain before second treatment with scrub engine	4.7×10^3	11	*Alternaria alternata* *Aspergillus niger* *Cladosporium herbarum* *Fusarium sporotrichioides* *Penicillium expansum* *Penicillium verrucosum*
Grain after hydrothermal treatment before the first crusher system	2.0×10^4	10	*Aspergillus flavus* *Aspergillus niger* *Fusarium sporotrichioides* *Penicillium expansum* *Penicillium verrucosum* *Rhizopus oryzae*
Flour	3.1×10^3	14	*Penicillium expansum* *Penicillium viridicatum* *Penicillium oxalicum* *Rhizopus oryzae*
Coarse bran	3.5×10^3	10	*Acremonium murorum* *Alternaria alternata* *Fusarium sporotrichioides* *Fusarium avenaceum*
Fodder flour	4.8×10^3	13	*Acremonium murorum* *Alternaria alternata* *Fusarium sporotrichioides* *Paecilomyces variotii* *Rhizopus oryzae*

During the hydrothermal treatment of grain in ripening containers after 10–12 hours, as humidity changed from 14 to 16%, *Aspergillus flavus*, *A. niger*, Fusarium sporotrichioides, *Penicillium verrucosum* micromycetes became more abundant. In flour, fungi of the *Penicillium* genus prevailed, while in coarse bran and fodder flour – *Alternaria alternata*, *Acremonium murorum*, and *Fusarium sporotrichioides* fungi were most abundant.

Mycological analysis of flour used for baking bread revealed the presence of micromycete propagules. In the bakery mill, in freshly milled rye flour most abundant were micromycetes of the *Penicillium* genus (*Penicillium biforme, P. brevicompactum, P. chrysogenum, P. cyclopium, P. expansum, P. roqueforti, P. velutinum*). Some of them can be considered as accidental, others should be regarded as potential producers of toxins and their distribution in the process of rye bread production should be monitored. In rye bread propagules of *Aspergillus flavus* and *Fusarium culmorum* fungi and *Bacillus subtilis* bacteria were recorded. In second-rate wheat flour taken from the sieve, *Aspergillus flavus*, *A. niger*, *Rhizopus oryzae* micromycetes, *Bacillus subtilis*, and other mucilaginous bacteria were recorded. In samples from rye flour sieve *Penicillium biforme*, *P. chrysogenum*, *P. roqueforti*, *Aspergillus flavus*, and *Ulocladium chartarum* micromycetes were also recorded.

Propagules of various micromycetes were also recorded in flour brought into bakery from other regions of the country. In unpacked first-rate wheat flour the micromycete diversity depended upon the locality of grain origin, time of milling, humidity and other factors. *Aspergillus repens* (=*Eurotium herbariorum*) (Wiggers) Link ex Gray), *Mortierella hyalina*, *Oedocephalum lacrimisporum*, *Penicillium biforme*, *P. chermesinum*. *P. chrysogenum*, *P. diversum*, *P. expansum*, *P. urticae* were isolated. In one pack of superfine flour kept in a mill storage area micromycete propagules of the following species were recorded: *Fusarium graminearum*, *F. culmorum*, *F. moniliforme*, *Aspergillus flavus*, *A. puniceus*, *A. penicilloides*, *Penicillium chrysogenum*, *P. commune*, *P. biforme*, *Geotrichum candidum*, *Mycelia sterilia*. In other packs, however, the micromycete species diversity and abundance were significantly lower. *Bacillus subtilis* bacteria were not recorded in this flour. In rye flour brought from other climatic zones of Lithuania and kept packed for a couple of weeks, together with *Penicillium biforme*, *P. chrysogenum*, *P. expansum*, *P. urticae* fungi, *Aspergillus flavus*, *Fusarium graminearum*, *F. moniliforme*, and *Cladosporium herbarum* micromycetes were recorded.

Mycological analyses of other bread ingredients revealed the following: in salt, sown on malt agar extract using standard methods, solitary *Aspergillus fumigatus*

Table 3. Micromycetes prevailing in air of mill and bakery premises (detection frequency >50%).

Sampling place	Identified species	Prevailing micromycete species
Outside. At mill entrance	15	*Alternaria alternata* (Fr.) Keissl; *Ascochyta graminicola* Sacc.; *Aureobasidium pullulans* (de Bary) G. Arnaud; *Cladosporium cladosporioides* (Fressen.) G.A. de Vries; *Fusarium graminearum* Schwabe; *Fusarium solani* (Mart.) Appel et Wollenw; *Phoma exiqua* Desm.; *Penicillium chrysogenum* Thom
Grain storage premises	20	*Alternaria alternata*; *Aspergillus awamori* Nakazawa; *Aspergillus niger* Tiegh.; *Cladosporium herbarum* (Pers.) Link ex Gray; *Mucor racemosus* Fresen.; *Penicillium biforme* Thom; *Penicillium chrysogenum*; *Ulocladium chartarum* (Preuss) E.G. Simmons
Flour storage premises	14	*Botrytis cinerea* Pers. et Fr.; *Cladosporium cladosporioides*; *Cladosporium herbarum*; *Fusarium moniliforme* J. Sheld.; *Penicillium chrysogenum*; *Penicillium expansum* Link; *Sclerotinia sclerotiorium* (Lib.) De Bary; *Ulocladium chartarum*
Milling unit in mill (air)	12	*Mortierella hyalina* (Harz) W. Gams; *Penicillium chrysogenum*; *Penicillium expansum*; *Penicillium spinulosum* Thom
Flour weighing and sifting premises of the bakery (air)	17	*Botrytis cinerea*; *Cladosporium herbarum*; *Exophiala jeanselmei* (Langeron) McGinnis et A.A. Padhye; *Fusarium graminearum* Schwabe; *Mucor circinelloides* Tiegh; *Penicillium biforme* Thom; *Penicillium chrysogenum*; *Penicillium verrucosum* Dierckx; *Rhizopus oryzae* Went ex Prins. Geerl.
Dough preparation premises of the bakery (air)	12	*Aspergillus awamori*; *Aspergillus clavatus* Desm.; *Aspergillus flavus* Link; *Aspergillus oryzae* (Ahlb.) Cohn; *Cladosporium cladosporioides*; *Cladosporium herbarum*; *Penicillium expansum*
Premises for dough preparation for baking (air)		*Aspergillus awamori*; *Aspergillus oryzae*; *Botrytis cinerea*; *Cladosporium herbarum*; *Fusarium graminearum*; *Penicillium expansum*; *Penicillium verruculosum* Peyronel
Bread baking premises (air)		*Aspergillus awamori*; *Aspergillus clavatus* Desm.; *Aspergillus flavus*; *Aspergillus oryzae*; *Cladosporium cladosporioides*; *Cladosporium herbarum*; *Penicillium expansum*
Premises of bread prepacking (air)	11	*Alternaria alternata*; *Aspergillus awamori*; *Aspergillus clavatus*; *Botrytis cinerea*; *Penicillium chrysogenum*; *Penicillium pallitans* Westling; *Penicillium verrucosum*

Table 4. Abundance of micromycetes in air of bread bakery and its surroundings, cfu m^{-3}.

Sampling place	Micromycetes	Bacteria	Yeasts
Outside. At mill entrance	27 185 ± 2017	–	–
Premises of grain storage in granaries	33 332 ± 4016	–	–
Flour storage premises	4 640 ± 80	–	–
Mill. At milling engines	8 280 ± 201	–	–
Mill. Between milling engines	6 520 ± 280	–	–
Premises for flour weighing and sifting	1 200 ± 40	38 579 ± 230	24 262 ± 3828
Premises for storage and treatment of indirect materials	1 015 ± 16	1 810 ± 10	53 ± 16
Dough preparation premises	970 ± 13	670 ± 70	55 ± 6
Premises for dough preparation for baking	1 020 ± 21	1 450 ± 10	85 ± 12
Bread baking premises (close to oven)	787 ± 34	2268 ± 628	219 ± 36
Premises for bread prepacking	873 ± 71	1 841 ± 128	36 ± 3

colonies were formed, while in starch syrup *Aspergillus awamori* species, which is close to *Aspergillus niger*, were recorded. Identification of these fungi is still a subject of discussion [46]. The number of micromycete species in caraway (*Carum carvi* L.) was considerably higher: *Aspergillus candidus*, *A. flavus*, *A. niger*, *Penicillium daleae*, *P. expansum*, and in food additive "Emipon" *Aspergillus awamori*, *A. fischeri*, *Candida albicans*, *Mycelia sterilia* were found. From calamus (*Acorus calamus* L.), on which some sorts of rye bread are baked, and which are kept in bread baking premises, some micromycetes were also isolated: *Alternaria alternata*, *Aspergillus niger*, *Aureobasidium pullulans*, *Mortierella isabellina*, *Mucor mucedo*, *Penicillium funiculosum*, *Mycelia sterilia*.

The packed leaven "Rekord", brought from Poland, and the leaven from Estonia produced only pure *Saccharomyces cerevisiae* cultures. On a plastic film for bread packing, which is kept in the raw materials preparation premises, *Cladosporium cladosporoides* and *Penicillium implicatum* micromycetes, as well as *Mycelia sterilia* were detected. Later these fungi were also recorded in other premises; they got on the ready bread and contaminated it.

In the premises and on the equipment of dough preparation micromycetes of the *Penicillium* genus prevailed (*P. chrysogenum*, *P. expansum*, *P. godlewskii*, *P. roquefortii*, *P. spinulosum*, *P. terrestre*), but *Cladosporium cladosporoides*, *Mycelia sterilia* were also isolated. From the formed dough loafs fungi of the *Aspergillus* genus were isolated; they were tentatively ascribed to the *Aspergillus awamori* Nakazawa species. The dough abounds in yeasts, mostly *Saccharomyces cerevisiae*. From the pulp of freshly baked rye bread single colonies of *Aspergillus cervinus* (Massee) Neill and *Penicillium brevicompactum* Dierckx were isolated. It confirms the statement that bread dough is a good insulating substance able to protect micromycete and bacterial propagules even when bread is baked at +252ºC temperature; it also proves that even bread baked at a high temperature is not free from the fungal and bacterial infections. This was also confirmed by the presence of single *Aspergillus oryzae* propagules in a freshly baked wheat bread. The slicing of bread also induces further contamination with micromycetes. On slicing equipment *Aspergillus oryzae*, *A. versicolor*, *A. repens*, (=*Eurotium herbariorum*), *Aspergillus* spp., *Absidia glauca*, *Penicillium biforme*, *P. paxilli*, *Cladosporium herbarum*, *C. resinae* (Lindau) G. A. de Vries (=*Amorphotheca resinae* Parbery), *Aureobasidium pullulans*, *Thelebolus erustaceus* (Fuckel) Kimbr., *Trichosporiella cerebriformis* (G.A. de Vries et Kleine-Notrop) W. Gams were recorded. Such abundance and species diversity of fungi of this area shows its connection with other premises and the outside air and reveals insufficient cleaning and maintenance of these premises and equipment (e.g., after application of oil on the equipment). This is demonstrated by the presence of *Amorphotheca resinae* fungi, which are known to assimilate oil fractions. The presence of *Thelebolus crustaceus* fungi confirms the contact of surrounding objects with excrement or fragments of upper skin layer. Frequent cleaning of bread slicing equipment considerably reduces its contamination with micromycete propagules, but did not completely eliminate it. On bread slicing knives, not cleaned for a day, *Penicillium biforme*, *P. palitans*, *Abidia glauca*, *Cladosporium resinae* propagules were recorded.

Prepacked and unpacked bread is stacked on wooden pallets, which, if insufficiently cleaned, are easily invaded by micromycete propagules and, therefore, become an infection source. *Alternaria alternata*, *Aspergillus flavus*, *Cladosporium cladosporoides*, *Penicillium commune*, *P. expansum*, *P. verrucosum* micromycetes, yeasts, and bacteria forming solitary colonies of limited size and mat colour were isolated from wooden pallets.

The bread is frequently sliced and packed while still hot and then in a cavity, which forms between the bread and packaging material, the humidity evaporating from hot bread accumulates and the bread surface becomes moist. Conditions favourable for fungal conidia or spores

Table 5. Abilities of micromycetes isolated from bread raw materials and their storage environment to synthesize toxic secondary metabolites (according to Cole *et al.*, T. 1–3, 2003) [10, 11].

Micromycete species	Isolated from	Synthesized toxin
Alternaria alternata	Calamus	Dehydroaltenusin; alternariol; altenuisol; altertenuol; alternusin; alternuic acid I, II, III; tenuazonic acid*.
Aspergillus candidus	Calamus, caraway	Candidusin A; kojic acid**; 3-nitropropionic acid**; xanthoascins**.
Aspergillus flavus	Calamus, caraway, second-rate wheat flour, rye flour	Ditryptophenaline; aflavine; aflavazole; paspaline; aflatrem; aflatoxin B_1; B_2; B_{2a}; B_3; G_1; G_2; G_{2a}; M_1; M_2; D_1; asportoxin.
Aspergillus fumigatus	Wheat flour, salt	Fumigaclavine A, B, C; fumitremorgin A, B, C; lonosulin; verruculogen; TR-2; tryptoquivaline A, B, C, D, E, F, G, H, I, J, K, L, M, N; fumigaclavine A, B, C; izofumigaclavine B; roquefortine; terreic acid*.
Aspergillus niger	First-rate wheat flour, calamus, caraway, rye flour	Ergosterol; malformin A*; xanthogmegnin*; aspergillins**; malformis**; neoechinulin A**; nigerazines**; nigragillin**; orlandin**; tubingensin A, B**.
Aspergillus oryzae	Wall plaster, wheat flour	Cyclopiazonic acid; kojic acid*.
Aspergillus penicilloides	First-rate and superfine wheat flour	Organic acids.
Aspergillus repens	Wall plaster in raw materials preparation premises, first-rate wheat flour	Prechinulin; asperflavin.
Aureobasidium pullulans	Calamus	Stigmasterol.
Botrytis cinarea	Superfine wheat flour	Fecosterol (C_{28} sterols); botrydial; dihydrobotrydial; botryaloic acid; botryaloic acid acetate; norbotryal acetate.
Fusarium culmorum	Rye flour	Cyclonerodiol; culmorin; izotrichodermin; T-2 toxin; HT-2 toxin; neosolaniol; solaniol; moniliformin.
Fusarium equiseti	First-rate wheat flour	Anguidine; scripenetriol; solaniol; isoneosoloniol; acuminatum; fusarenon-X; nivalenol monoacetati; fusarenon.
Fusarium graminearum	Superfine wheat flour	Culmorin; isotrichodermin; nivalenol; 4-deoxynivalenol; Rd toxin; vomitoxin; 3-acetyl-4-deoxynivalenol; fusacenon X1; nivalenol monoacetate; fusarenon; moniliformin.
Fusarium moniliforme	Superfine wheat flour	Fumanisin B_1, B_2, B_3, B_4, A_1, A_2, C_1, C_3, C_4; 8-0-methyl-javanicin; 8-0-methylsolaniol; nophtaguinones; macrofusin; moniliformin.
Penicillium biforme	Wall plaster, first- and second-rate wheat flour, rye flour	Rugulovasine A, B; cyclopiazonic acid.
Penicilium brevicompactum	Rye flour	Brevianamide C, D, F.
Penicillium chrysogenum	Wall plaster, second-rate wheat flour, rye flour	Cyclopiazonic acid; penicillic acid; ochratoxin A*; patulin*.
Penicillium cyclopium	Rye flour	Patulin; claviformin; clavitin; expansion; penicidin; mycoin; leucopin; tercinin; penicillic acid; moniliformin.
Penicillium daleae	Wall plaster, calamus, caraway	Kojic acid.
Penicillium expansum	Wall plaster, calamus, caraway	Roquefortine; roquefortine C; patulin; claviformin; clavitin; clavicin; expansion; penicidin; mycoin; leucopin; tercinin.
Penicillium funiculosum	Calamus	Funiculosin.
Penicillium roquefortii	Sifted rye flour	Roquefortine; roquefortine A, B, C; isofumigaclavine A, B; marcfortine A, B, C; patulin; claviformin; clavitin; clavicin; expansion.
Penicillium urticae	Rye flour	Patulin; claviformin; clavitin; clavicin; expansion; penicidin; mycoin; leucopin.
Penicillium verrucosum	Wheat grain	Ochratoxin A*; penicillic acid*; citrinin*; verruculogen*.
Penicillium viridicatum	Ceilings, wall plaster	Moniliformin; penicillic acid*; citrinin*; verruculogen*.
Phoma exiqua	Barley grain	Cytochalasin A, B; phomin; deoxaphomin; proxiphomin.
Rhizopus stolonifer	First- and second-rate wheat flour	Episterol.
Trichothecium roseum	Ceilings, wall plaster, rye flour	Crotocin; antibiotic T; trichothecolone; trichothecinol A, B, C; trichodiene; cyclonerodiol.

* Roth *et al.*, 1990 [85], ** Chełkowski, 1991[8].

to germinate, and for mycelium to spread further on the bread surface form. Micromycete colonies, most frequently of the *Penicillium*, *Aspergillus*, *Mucor*, *Rhizomucor*, *Rhizopus*, etc. genera, form on the bread.

Sometimes, sellers return large amounts of bread to the bakery. It should be properly maintained and not become the contamination source. From wheat buns seasoned with sesame, with an unbroken package, the following micromycetes were isolated: *Arthrinium sporophleum*, *Aspergillus flavus*, *A. repens* (*=Eurotium herbariorum*), *Penicillium biforme*, *P. expansum*, *P. oxalicum*, *P. roqueforti*, *P. spinulosum*, *Wardomyces anomalus* F.T. Brooks & Hansf., *Mycelia sterilia*. The bun is a multicomponent product; therefore, it is affected by a great variety of micromycete species. *Penicillium expansum*, *P. roqueforti*, and *P. viridicatum* were isolated from rye bread packed into plastic film with holes. All these micromycetes are potential producers of mycotoxins [24]. The contaminated bread should not be brought back to the territory of the bakery because it can become the source of micromycete contamination of the production premises and freshly baked bread. People engaged in bread production should not be involved in the utilization of the spoiled bread as they can become distributors of fungi contaminating fresh bread.

Micromycete diversity on food-stuffs in the bakery was also predetermined by mycological conditions of production premises; the data on their abundance is presented in Table 3.

From the samples of air, wall and ceiling scrapings in utility room *Ulocladium chartarum*, *Geotrichum candidum*, *Geomyces pannorum*, *Arthroderma tuberculatum*, *Cladosporium cladosporoides*, *C. herbarum*, *Penicillium viridicatum*, *Alternaria alternata*, *A. tenuissima*, *Chrysosporium farinicola*, *Myceliophthora vellerea*, *Botryotrichum piluliferum*, *Mortierella isabellina* micromycetes were recorded. Closer to ovens *Aspergillus awamori*, *A. niger*, *A. flavus*, *Penicillium biforme* were more abundant. From plaster bits fallen from walls *Aspergillus ochraceus*, *Circinella circinans*, *Aspergillus repens* (*=Eurotium herbariorum*), *Penicillium chrysogenum*, *P. expansum*, *P. viridicatum* were isolated. From plaster bits 4 bacteria species, including *Bacillus subtilis*, which is hazardous to bread, yeasts *Saccharomyces cerevisiae*, *Candida albicans*, and *Pichia fermentans*, as well as *Mucor racemosus*, *Olpitrichium macrosporum*, *Penicillium biforme*, *P. chrysogenum*, *P. daleae*, *P. expansum*, *Ulocladium chartarum*, *Mycelia sterilia* micromycetes were isolated. Micromycetes developing on walls, ceilings, equipment, and other surfaces are rather hazardous contaminants of the bread production process because micromycete propagules get not just on the raw material, the ready bread, but also impose risk to the staff. Data on the micromycete distribution in the air of the bakery premises is presented in Table 4.

In order to evaluate potential hazard that can be caused by the above-mentioned micromycetes, it is essential to investigate the data on their abilities to synthesize secondary metabolites listed in Table 5.

The data presented in Table 5 shows that among micromycetes recorded on raw materials, fungi able to spread actively and synthesize toxins of various natures are present. These fungi can get into bread before or after baking. In wheat grain intended for flour production the recorded amount of deoxynivalenol (DON) reached 0.1 mg/kg. At that moment no other toxins were recorded. The same amount of DON was recorded after the first shelling with a scrub engine as well as in bran and fodder grain. DON was not recorded in flour. It can be concluded that mycotoxin deoxynivalenol accumulates in seed-coat and, therefore, it gets into fodder grain or bran, but into flour.

Climatic conditions of Lithuania are favourable for the fungi of the *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria*, *Drechslera*, *Cladosporium*, *Curvularia*, *Acremonium* genera to spread on grain of cereals and pulses. As already mentioned, many micromycetes recorded on grain are able to synthesize mycotoxins hazardous to humans and animals. Recently, abundant data on the carcinogenic

Table 6. Micromycetes isolated from raw material of plant origin before and after extrusion.

Sample	Before extrusion	After extrusion
Mixture of wheat, barley, pea, maize flour	*Aspergillus oryzae, Cladosporium herbarum, Drechslera sorokiniana, Fusarium avenaceum, F. culmorum, F. solani, F. sporotrichioides, Penicillium piceum, P. verrucosum, P. verruculosum, Rhizopus oryzae Mycelia sterilia*	*Acremonium* spp., *Cladosporium herbarum, Fusarium avenaceum, F. culmorum, F. equiseti, F. graminearum, F. poae, F. sporotrichioides, Penicillium expansum, Mycelia sterilia*
Maize flour	*Absidia spinosa, Aspergillus amstelodami, A. oryzae, A. penicilloides, A.* (*Eurotium*) *repens, A. versicolor, Mucor silvaticus, Penicillium expansum, P. variabile, P. verrucosum, Rhizopus oryzae, Mycelia sterilia*	*Aspergillus flavus, A.oryzae, Mucor racemosus, Penicillium verrucosum, P. melinii, Rhizopus oryzae, Mycelia sterilia*
Barley flour	*Alternaria alternata, Drechslera sorokiniana, Fusarium poae, Fusarium* spp., *Acremonium kiliense, Aspergillus fumigatus, Fusarium oxysporum, F. avenaceum, F. moniliforme, F. sambucinum, F. tricinctum, Monilia* spp., *Mucor* spp., *Oidiodendron maius, Penicillium rugulosum, P. tardum, P. verrucosum, P. viridicatum, Rhizopus oryzae, Trichothecium roseum, Mycelia sterilia*	*Alternaria alternata, Drechslera sorokiniana, Fusarium poae, Fusarium* spp., *F. culmorum, F. tricinctum, Aureobasidium pullulans, Cladosporium herbarum, Penicillium corylophilum, Verticillium album, Mycelia sterilia*

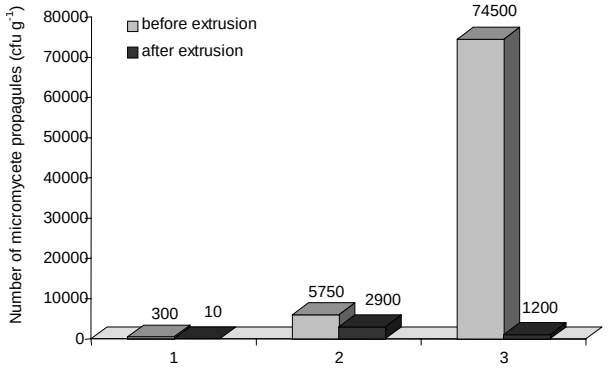

Figure 2. Abundance of micromycetes during different extrusion phases: 1 – Mixture of wheat, barley, pea, maize before the extrusion humidity 12.92% and after extrusion – 7.94%; 2 – Barley flour before the extrusion humidity 10.76% and after extrusion – 4.58%; 3 – Maize flour before the extrusion humidity 13.21% and after extrusion – 6.43%.

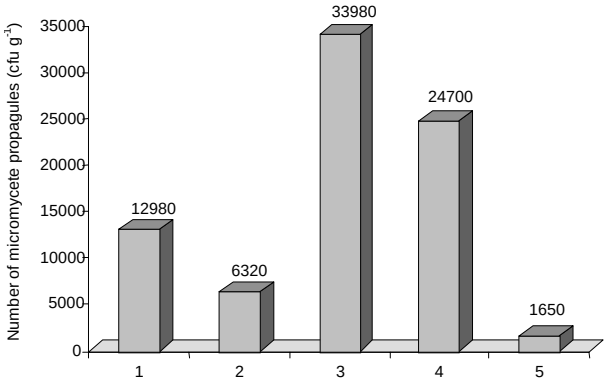

Figure 3. Changes in micromycete propagules during various technological stages of groats production: 1 – barley grain; 2 – barley after first shelling; 3 – bran after first shelling; 4 – bran after second shelling; 5 – barley groats.

action of mycotoxins has appeared [99]. In the course of yield handling, transporting, and especially processing, micromycetes developing on the grain surface easily get into flour, groats, and other products. It was noticed that the number of micromycete propagules in flour and groats depended upon the amount of seed-coat particles in them. Therefore, during technological processes, grain contaminated with micromycetes is cleaned. Many common operations are performed during groats production from various grains. For example, grain intended for the production of barley and buckwheat groats are dried, cleaned, sorted according to the size. Cleaned and sorted grain is shelled in the scrub engines; their emery surface removes grain seed-coat together with all contaminants and partially micromycetes. The data on the abundance of micromycetes on various plant origin raw materials before and after extrusion is presented on Figure 2.

The data of Figure 2 shows the impact of the extrusion upon the abundance of micromycetes on the mixture of wheat, barley, pea, and maize (1); barley flour (2); maize flour (3). The contamination with micromycete propagules is mostly reduced after the extrusion of maize kernels. This is due to the fact that in the extruder, crushed grain is being affected by 1.4–1.5 MPa pressure and frictional force; it warms up to 180ºC and turns into a homogeneous mass. The process of extrusion lasts for about 30 seconds. As the product leaves the extruder, the pressure reduces to the atmosphere level, water present in the tissues starts to boil intensively, the inner "burst" of the product takes place, the whole mass bulges, and the tissue structure changes. Due to intensive boiling and water evaporation, the humidity amount in the product decreases up to 50%. Under the impact of pressure and temperature, some micromycetes perish; the activity of others is considerably reduced. Only micromycetes resistant to these physical factors survive.

Changes in the micromycete species diversity occurring during the extrusion are presented on Table 6.

After extrusion, in the mixture of wheat, barley, pea, and maize fungi ascribed to the *Fusarium* genus (*F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. sporotrichioides*) producing toxins of the trichotecene group survived together with *Penicillium expansum*, which produces roquefortine and patulin. In maize flour *Aspergillus flavus*, producing aflatoxin B_2, and in barley flour *Alternaria alternata*, producing alternariol and tenuazonic acid, as well as *Fusarium tricinctum*, producing T-2 toxin, were recorded. Investigation of the alterations of mycological state of barley grain intended for groats production during various technological stages of the processing were revealed (Fig. 3 and Tab. 7).

In barley grain intended for the production of groats the number of micromycete propagules was 12.98×10^3 cfu g^{-1}. After the first shelling in the scrub engine the number of micromycete propagules in grain was reduced by half, and in barley groats after the second shelling the number of micromycete propagules was reduced to 1.65×10^3 cfu g^{-1}. It can be concluded that removal of the seed-coat from barley grain was crucial for the reduction in the abundance of micromycete propagules. In barley bran the numbers of micromycete propagules after the first (33.9×10^3 cfu g^{-1}) as well as after the second (24.7×10^3 cfu g^{-1}) shelling were considerably higher than in groats.

Data on micromycete species diversity and its alteration in barley mass during various technological stages of groats production is presented in Table 7. It should be mentioned that species diversity recorded in barley mass only slightly changed during all stages of processing. Micromycetes of some species were recorded in all barley mass samples during all technological stages of groats production. These were *Alternaria alternata*, *Cladosporium herbarum*, *Drechslera sorokiniana*. From barley intended for groats production and from freshly produced groats *Alternaria alternata*, *Cladosporium herbarum*, *Drechslera sorokiniana*, *Fusarium culmorum*, *F. graminearum*, *F. poae*, *F. sporotrichioides*, *Verticillium album*, *Mycelia sterilia* were isolated. That time the level of contamination was reduced from 12.98×10^3 cfu g^{-1} to 1.65×10^3 cfu g^{-1}. Detection of various micromycete species in the processed mass of plant origin can be partly explained by wide distribution of the mentioned micromycete propagules in the surrounding of the performed

technological processes: premises air, on equipment, tools, packages, and the staff. This is confirmed by the presence of *Penicillium* fungi in barley after the first shelling, which were not isolated from the barley grain. Meanwhile, in the air samples of the production premises these fungi dominated.

During the production of buckwheat, different from the barley groats production, after the first removal of impurities, hydrothermal treatment of buckwheat grain was performed: vaporization, drying, and cooling (Fig. 4).

During vaporization of buckwheat grain, 5 minutes at a steam pressure of 0.25–0.30 MPa and 110–140ºC temperature, microorganisms partly perish, and the functioning of others is suppressed. After that, buckwheat grain is kept for 30 minutes in granaries so that moisture could distribute evenly in the whole buckwheat mass. Buckwheat grain is dried in contact vapour driers. Under such conditions the survived microorganisms recover and get a new impulse to function. Hydrothermal treatment of buckwheat grain increases the coefficient of grain shelling. Therefore, in the environment of micromycete development the amount of easily available nutritious materials increases. In buckwheat mass, however, the abundance and species diversity of micromycetes considerably changed. The contamination of buckwheat grain brought into the storehouse was $48.0 \pm 10.2 \times 10^3$ cfu g^{-1} of micromycete propagules, after hydrothermal treatment with vaporizer it made only $0.1 \pm 0.06 \times 10^3$ cfu g^{-1}. On locally grown grain *Alternaria alternata*, *Chaetomium globosum*, *Fusarium semitectum*, *Penicillium olsonii*, *P. palitans*, *Rhizomucor pusillus*, *Exophiala jeanselmei* fungi were abundant. After the hydrothermal treatment *Aspergillus* fungi (*A. candidus*, *A. carneus*, *A. cervinus*, *A. flavus*) became dominant. It should be mentioned that *A. candidus* fungi were recorded on these grains during all stages of their processing and groats production (Tab. 8, Fig. 5).

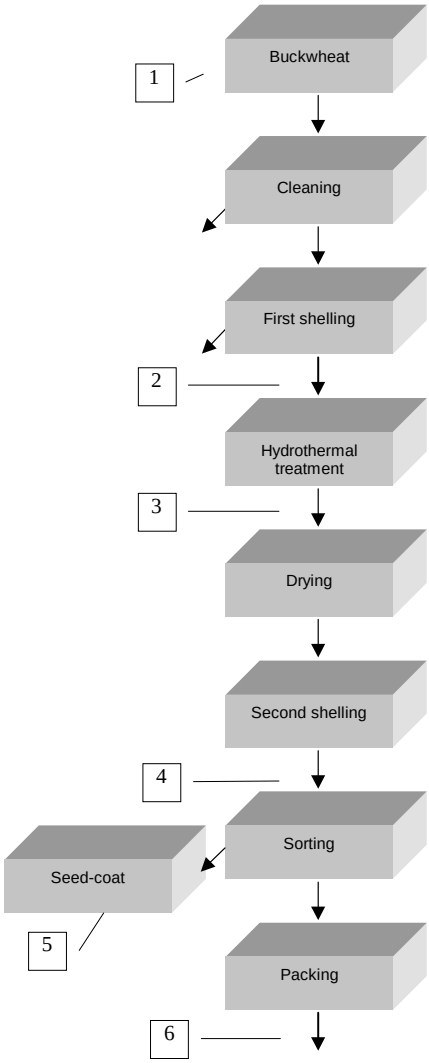

Figure 4. Technological scheme of buckwheat groats production and sampling places (1, 2, 3, 4, 5, 6).

Table 7. Micromycete diversity in barley mass during different technological stages of groats production.

Sample	Micromycetes isolated from the barley mass
Barley grain intended for groats production. Storehouse	*Acremonium kiliense, Alternaria alternata, A. radicina, Aspergillus penicilloides, Cladosporium herbarum, Drechslera sorokiniana, Fusarium poae, F. avenaceum, F. culmorum, F. equiseti, F. graminearum, F. tricinctum, F. moniliforme, F. sporotrichioides, Mortierella hyalina, Mucor hiemalis, Verticillium album, Mycelia sterilia*
Barley after the first shelling with scrub engine	*Alternaria alternata, Cladosporium herbarum, Drechslera sorokiniana, Fusarium avenaceum, F. proliferatum, F. semitectum, F. sporotrichioides, Monilia* spp., *Mortierella lignicola, Mucor luteus, Penicillium corylophilum, P. verrucosum, P. chrysogenum, P. corymbiferum, P. expansum, P. oxalicum, P. decumbens, Mycelia sterilia*
Barley bran after the first shelling with scrub engine	*Acremonium strictum, Alternaria alternata, Aspergillus niveoglaucus, Cladosporium cladosporioides, C. herbarum, Drechslera sorokiniana, Fusarium avenaceum, F. culmorum, F. poae, F. oxysporum, F. tricinctum, Mucor racemosus, Oidiodendron maius, Rhizopus oryzae, Mycelia sterilia*
Barley bran after the second shelling with scrub engine	*Alternaria alternata, Aspergillus versicolor, Cladosporium herbarum, Drechslera sorokiniana, Fusarium avenaceum, F. graminearum, F. sambucinum, F. solani, F. sporotrichioides, Oidiodendron maius, Penicillium claviforme, P. verruculosum, Sporotrichum aurantiacus*
Barley groats	*Alternaria alternata, Cladosporium herbarum, Drechslera sorokiniana, Fusarium culmorum, F. graminearum, F. poae, F. solani, F. sporotrichioides, Penicillium expansum, Penicillium* sp., *Verticillium album, Mycelia sterilia*

Their growth and development is particularly intensified some time after hydrothermal treatment. Under favourable conditions these fungi can produce and excrete various toxic substances: citrinin, kojic acid; when they grow together with *Aspergillus parasiticus* they produce aflatoxins, sometimes synthesize terphenylin, xanthoascin, 3-nitropropionic acid. If *A. candidus* develop on food products, these toxins increase the risk of diseases, allergy, and poisoning [51, 55, 60]. After cultivation of hydrothermically treated buckwheat single colonies of *Aspergillus flavus*, *A. carneus*, and *A. cervinus* developed; these fungi are also reported as active producers of toxic secondary metabolites.

On freshly produced buckwheat groats only single colonies of *Aspergillus candidus*, *A. cervinus*, some *Penicillium fungi* (*P. palitans*, *P. nalgiovense*, *P. meleagrinum*) were recorded; the contamination made only $0.05 \pm 0.01 \times 10^3$ cfu g^{-1}.

Larger amount of *Aspergillus candidus* propagules accumulated in by-products formed of buckwheat seed-coats; here *Aspergillus oryzae*, *Fusarium equiseti*, *F. graminearum*, *F. semitectum*, *F. solani* micromycetes were recorded.

Contamination of coarse buckwheat groats on sale did not exceed $0.1 \pm 0.03 \times 10^3$ cfu g^{-1}. *Aspergillus candidus*, *A. cervinus*, *A. niger*, *Penicillium digitatum*, *P. meleagrinum*, *Rhizomucor pusillus*, *Mycelia sterilia* were isolated from coarse buckwheat groats packed in cardboard. From the sanitary point of view, more interesting are the micromycetes detected during all stages of groats production because they are constantly consumed for food. Temperature resistance enables micromycetes to survive and function under extreme conditions that form in the course of food-stuffs production.

Contamination of fine buckwheat groats on sale did not exceed $0.02 \pm 0.01 \times 10^3$ cfu g^{-1} of micromycete propagules. *Aspergillus cervinus*, *A. flavus*, *Candida* spp., *Cladosporium sphaerospermum*, *Penicillium digitatum*, *P. nalgiovense*, *P. palitans*, *Rhizomucor pusillus*, *Rhizopus oryzae*, *R. stolonifer*, *Mycelia sterilia* were isolated.

In the premises of groats production (in the air, dust, and by-products) *Aspergillus niger*, *A. oryzae*, *Exophiala jeanselmei*, *Penicillium digitatum*, *P. nalgiovense*, *P. palitans*, *P. stoloniferum*, *Rhizomucor pusillus*, *Rhizopus oryzae*, *Mycelia sterilia* dominated. In buckwheat seed-coat *Aspergillus candidus*, *A. oryzae*, *Fusarium equiseti*, *F. graminearum*, *F. semitectum*, *F. solani*, *Rhizopus oryzae*, *Mycelia sterilia* prevailed. Therefore, shelling efficiently reduces the amount of toxin-producing micromycete propagules. It is important that the least possible amount of micromycete propagules from the environment re-enter the pre-cleaned raw material.

The ability of the isolated micromycete strains to synthesize certain toxins was investigated: *Aspergillus flavus* strains isolated from fine barley and buckwheat groats on sale synthesize aflatoxin B_2; *Drechslera sorokiniana* strain isolated from barley synthesizes sterigmatoxin and cytochalasin; *Aspergillus clavatus* strain isolated from buckwheat synthesizes patulin; *Penicillium palitans* strain isolated from barley and wheat and *Penicillium verrucosum* strain isolated from rye synthesize penitrems A and B.

Some *Alternaria alternata* strains synthesize tenuazonic acid and altenuen. Majority of *Fusarium graminearum*, *F. poae*, *F. sporotrichioides* strains isolated from grain and flour are able to synthesize trichothecenes A, but with different intensity. Trichothecenes B are synthesized by some *Fusarium graminearum*, *F. culmorum*

Table 8. Micromycetes isolated from buckwheat mass during different technological stages of groats production.

Sample	Contamination with micromycetes (cfu $g^{-1} \times 10^3$)	Isolated micromycete species
Buckwheat grainRaw material Storehouse	48.0 ± 10.2	*Alternaria alternata, Aspergillus oryzae, A. candidus, A. wentii, Chaetomium globosum, Exophiala jeanselmei, Fusarium semitectum, Geotrichum candidum, Mucor circinelloides, Mucor silvaticus, Penicillium digitatum, P. expansum, P. olsonii, P. palitans, Rhizomucor pusillus, Rhizopus oryzae, Mycelia sterilia*
Buckwheat after first shelling with an engine	3.1 ± 0.2	*Alternaria alternata, Aspergillus oryzae, A. candidus, Cladosporium cladosporioides, Fusarium equiseti, F. poae, Mucor* spp., *Penicillium commune, P. digitatum, P. palitans, Rhizomucor pusillus, Rhizopus oryzae, R. stolonifer, Mycelia sterilia*
Buckwheat after second shelling with an engine	1.9 ± 0.2	*Aspergillus oryzae, Penicillium digitatum, P. palitans, P. citrinum, P. commune, Rhizomucor pusillus, Rhizopus stolonifer*
Buckwheat after hydrothermal treatment	0.1 ± 0.06	*Aspergillus candidus, A. carneus, A. cervinus, A. flavus, Cladosporium sphaerospermum, Penicillium meleagrinum, P. nalgiovense, Rhizopus oryzae, Mycelia sterilia*
Freshly produced buckwheat groats	0.05 ± 0.01	*Aspergillus candidus, A. cervinus, Penicillium meleagrinum, P. nalgiovense, P. palitans, Mycelia sterilia*
Buckwheat seed-coat from shelling engine	1.42 ± 0.25	*Aspergillus candidus, A. oryzae, Fusarium equiseti, F. graminearum, F. semitectum, F. solani, Rhizopus oryzae, Mycelia sterilia*

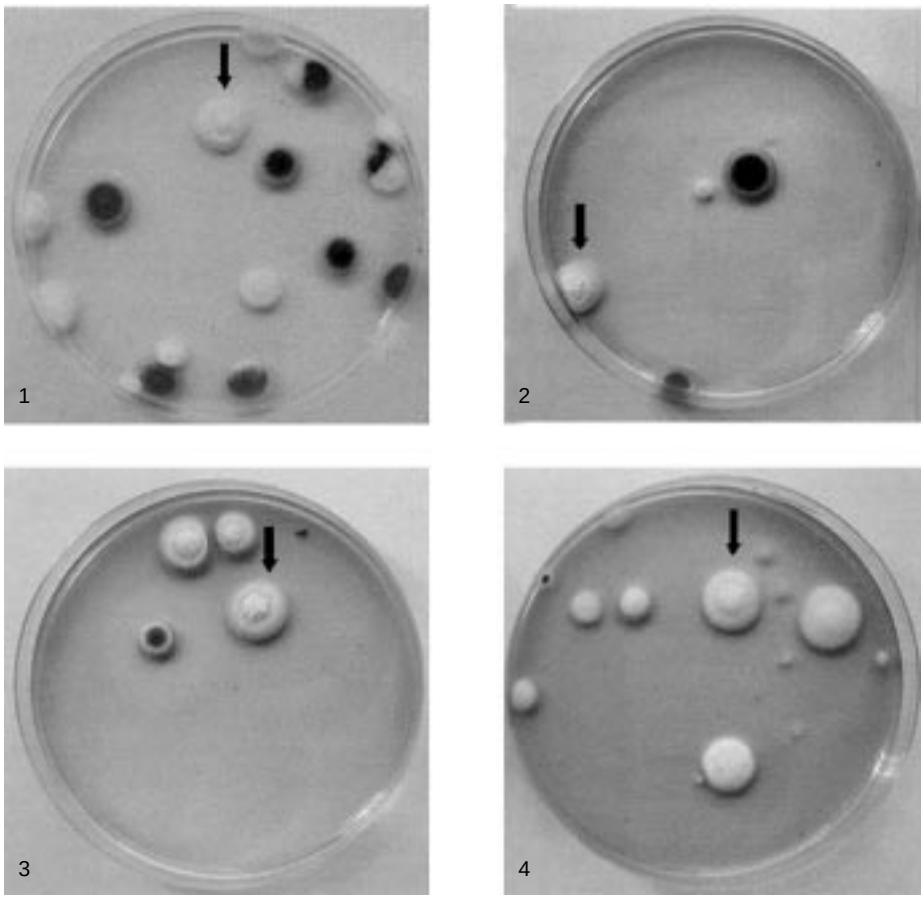

Figure 5. Contamination of buckwheat mass with micromycetes during various technological stages: 1 – buckwheat grain; 2 – grain after vaporization; 3 – freshly produced buckwheat groats; 4 – buckwheat groats on sale. ↓ – *Aspergillus candidus* micromycetes recorded during subsequent technological stages.

strains. Many of them isolated from grain also synthesize zearalenon. This confirms the opinion of other researchers [68].

Therefore, micromycetes and mycotoxins produced and excreted by them are important ecological factors that can determine the food quality, and through slow but constant action exert serious risk to human health.

Together with the researchers of the Institute of Biochemistry [4] investigations dealing with the clarification of the impact *in vivo* and *in vitro* of toxins ochratoxin (OTA) and patulin (Pt), synthesized by *Penicillium* fungi, upon Balb/c clone mice were performed. Investigations on the impact of the selected *Penicillium variabile* strain on mice getting it *per os* were performed; the experiment lasted for 28 days. Changes in blood plasma and ratio of its cell elements of mice in the experimental group were recorded, by 20% lower body weight of the tested mice, comparing with the control, was registered. For the analysis of mycotoxin action *in vitro* three cell lines of blood origin were selected: human promyelocytic leukemia HL60, chronic myelogenic leukemia K562, and pre-B leukemia REH cells. As the cells were affected by different concentrations of the investigated mycotoxins, it was revealed that the toxic impact of patulin in all cases was stronger than that of ochrotoxin A. However, the process of apoptosis in the tested cell cultures was more actively induced by ochrotoxin A. The most resistant to the impact of these toxins were myelogenic K562 cells.

This example enables consideration of each micromycete strain characterized by toxicity, isolated from food raw materials and products, as a collection of toxins. As such, a set entering the human or animal organism can affect various organs or their systems; the action of the toxins can sum up or go into separate directions. Results of the investigations of blood nature cells *in vitro* showed their different sensitivity to the impact of OTA and Pt, which can manifest in the organism, not just in impediment of proliferation but also in cell apoptosis and necrosis.

CONCLUSIONS

1. While choosing raw materials of plant origin intended for the production of food-stuffs, it is essential to consider their contamination not just with physical particles (glass, wood, metal), dead grain parasites, chemical pollutants (heavy metals, pesticides, fertilizers), but also microbial contamination, which is often less apparent. Under favourable conditions, however, it can

become really hazardous due to functional activity, the synthesized metabolites, and particularly high diversity of the excreted toxins.

2. Microorganisms get on raw materials and food-stuffs in different ways and at different moments. The grain and seed maturing phase can be regarded as the beginning of microbial contamination. Later, there follows grain harvesting, storage, transportation of intermediate products and ready production by screw transporters or conveyor belts, packing of the ready products, and storage of the packed production. During all these phases, under the influence of abiotic and biotic factors, grain can be contaminated with various microorganisms.

3. Grain and their products are sensitive to the damage caused by micromycetes, especially when humidity of grain and the surrounding environment increases and the temperature (18–28ºC) is favourable for their development. When the thermoenergetical regime in a grain heap is disturbed, physiological, biochemical, and microbiological processes are strengthened; various, frequently toxic and, therefore, hazardous to human health, metabolites are more intensively produced.

4. In the course of raw material processing, as the raw material is hydrothermically treated, a large portion of micromycetes are eliminated. The activities of micromycetes, which are able to survive and function under conditions of high temperature (*Aspergillus flavus*, *A. niger*, *A. cervinus*, *A. candidus*, *A. oryzae*, *A. fumigatus*, *Penicillium brevicompactum*, *P. viridicatum*, *P. palitans*, *Rhizopus oryzae*, *R. stolonifer*, *Rhizomucor pusillus*), are intensified, the amount of the excreted toxins increases.

5. Micromycetes isolated from cereal grain, flour, bread, groats are identified as producers of the following toxins: aflatoxin B_2, ochratoxins A and B, patulin, citrinin, roquefortine C, claviformin, paxilin, cytochalasines, penitrems A and B, sterigmatocystine, alternariol, altertenuol, tenuazonic acid, moniliformin, trichotecene A, trichothecene B, zearalenon, kojic acid, cyclopiazonic acid, T-2 toxin, deoxynivalenol.

6. The research results allow the statement that micromycetes and their toxic secondary metabolites isolated from cereal grain, raw material, and ready products are important factors determining the quality and safety of widely consumed products and, therefore, they should be constantly controlled and regulated.

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