EKOLOGIJA. 2006. Nr. 3. P. 105–111

Mycotoxin producents in the grain layer

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Micromycetes not only deteriorate the quality of grain, but also are potential producents of mycotoxins. The most reliable way not to contaminate grain with mycotoxins is to avoid their rise. Therefore, reducing the contamination of food products with mycotoxins, precautionary means pointed against mycotoxins are very important. Low moisture is the most significant element to reduce the vitality of mycotoxins. It is really important to choose a proper regime of drying and to desiccate grain before mould fungi have damaged it. The present research analysed the drying of wheat, malty and fodder barley in a mixed-flow grain dryer and in an active ventilation crop-bin. The influence of these drying technologies on the mycological contamination of grain was estimated.

Key words: grain, drying, micromycetes

INTRODUCTION

Nowadays more and more attention in the world is paid to the control of mycotoxins in food products. Mycotoxins are chemical combinations that are dangerous for human and animal health and can cause mycotoxicosis as well as cancer (Abramson, 1998; Humpisch, 2001; Kent, Evers, 1994). Their toxicity depends not only on the amount but also on the frequency of their getting into an organism. Even small amounts of mycotoxins repeatedly getting into an organism for a long period of time can be risky for health (Abramson, 1998; Bakutis, 2004; Karppanen et al., 1985; Moss, 1991).

The majority of mycotoxins are chemically tough, resistant to temperature, conditions of storage and processing technologies. Therefore, detoxication is not always effective. In addition, during the process of detoxication, substances which themselves are dangerous for health may be employed. The most reliable way to avoid the contamination of food products with mycotoxins is to eliminate the causes of their rise (Bakutis, 2004; Bartels, Rodemann, 2003; Kells et al., 2001; Ryden et al., 2003; Scott, 1991). Thus, in reducing the contamination of food products with mycotoxins, precautionary means pointed against mycotoxin producents – micromycetes – are very important.

Micromycetes easily adapt to the changeable environmental conditions, colonize and assimilate a great deal of substrata. Therefore, grain, containing various nutritive substances, makes a very good environment for the development of mould fungi which reduce the nutritive value and viability of grain and can become the cause of self-heating (Bakutis, 2004; Humpisch, 2001; Jayas, 1995; Karppanen et al., 1985; Lugauskas et al., 2002). Moreover, grain gets a specific smell. The grain colour changes as some micromycetes produce and contaminate it with poisonous substances – mycotoxins. Dioxinivalenol (DON), zearalenon (ZEA), ochratoxin (OTA), aphlatoxin and fumonizines are mycotoxins most frequently found in grain (Abramson, 1998; Krasauskas, 2005; Kuzmienė et al., 1991; Lacey, Magan, 1991; Moss, 1991; Scott, 1991).

The propagules of mould fungi, frequently found in grain and its surroundings, start to develop when favourable conditions emerge. Temperature and substratum moisture are the most significant factors that determine the intensity of micromycete development and grain damage (Abramson, 1998; Humpisch, 2001; Jayas, 1995; Lacey, Magan, 1991). In August, when the biggest part of grain harvest is stored up, the average day temperature is 22.4 ± 3.3 ˚C. Such a temperature is optimal or very close to the optimal for the development of fungi of the genera *Alternaria*, *Cladosporium*, *Fusarium* and *Penicillium* which prevail in grain. Thus, in Lithuanian climactic conditions the substratum moisture has a crucial influence on the vitality of mould fungi in the time of grain harvesting. Long-term research of grain delivered to processing corporations showed that in Lithuania the moisture of about 91% of flailed grain is more than 15% (Novošinskas, 1999), i. e. favours the development of micromycetes. Therefore, it needs to be immediately dried or conserved in some other ways.

Drying is the most popular way of grain harvest conservation, in which grain is dried up to the critical moisture: grain of ear crops up to 14% and oleaginous seeds of plants up to 9%. Low moisture in grain is the

most significant factor to protect it from mycological contamination. Not fully dried grain or dried slowly can start to mould and get damaged. Therefore, it is very important to choose the proper drying regimes and to dry grains before mould fungi have done harm to them (Bartels, Rodemann, 2003; Bruce, Ryniecki, 1991; Kent, Evers, 1994; Kuzmienė et al., 1991; Kröll, 1989; Lacey, Magan, 1991; Obst et al., 2000).

The aim of the present study was to explore the influence of drying regimes on the mycological contamination of grain harvest.

MATERIALS AND METHODS

In practice, grain is usually dried in mixed-flow grain dryers or in crop-bins of active ventilation. These drying technologies differ in the drying agent's temperature, ventilation intensity and the condition of the dried grain layer. While drying grain harvest, it is crucial to reduce the vitality and number of mould fungi. Thus, the ways of drying are compared in relation to units that form the variation of micromycete colonies (cfu) in the grain mound.

Grain drying was studied on farms in Kaunas district in 2004. The 'Astron' species of wheat dried in a 'Cimbria' mixed-flow dryer (fecundity 20 t/h) was explored. Grain drying was monitored on four different days – August 25, September 3, 8 and 13 (on that day two drying cycles were explored). Therefore, the moisture of the dried grain was different but the temperature of the drying agent was always the same (90 °C). We fixed grain moisture and the amount of micromycetes in the grain before and after drying. Also, ALMEMO sensors ZA 9020-FSK (error of temperature measuring devices ±0.1 °C) and FH A646-21 (error of temperature measuring devices ±0.1 °C, error of relative humidity ±2%) were used to measure the temperature of the environment and grain after drying in a flow dryer.

The winter wheat 'Širvinta', malty barney 'Barkė' and fodder barley 'Henni' with the original moisture levels respectively 16.0 ± 0.053%, 18.7 ± 0.096% and 17.9 ± 0.071% were used. Their mycological contamination before drying is shown in Table 1.

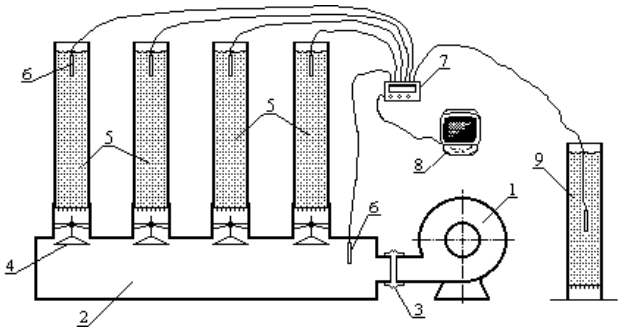

Fig. 1. Principal scheme of drying jig: *1* – ventilator, *2* – chamber of constant static pressure, *3* – flexible joint, *4* – valve, *5* – ventilated cylinders filled with grain, *6* – temperature and humidity sensors, *7* – secondary ALMEMO meter, *8* – computer, *9* – natural ventilation cylinder filled with grain

The experiments were carried out at the Lithuanian University of Agriculture in a specially designed jig (Fig. 1) consisting of an efferent ventilator and four cylinders connected with a constant static pressure camera by means of a divided joint. Each cylinder 0.18 m in diameter and 1.2 m in height contained 22.5 kg of wheat, 17.7 kg of malty or 17.8 kg of fodder barley. In all cylinders, grain was dried at the same time and the parameters of the drying agent were the same; only the intensity of ventilation was different. Before each drying it was regulated with a valve.

We fixed the mass of grain, the duration of drying, the surrounding temperature, the temperature of the blown out air by ventilator 1 and the relative humidity in the chamber of constant static pressure 2 and in the upper grain mound layers. The air temperature and relative humidity were measured using ALMENO sensors FH A646-21. The measurement results were entered every 10 minutes in the secondary instrument ALMEMO 3290. The grain was ventilated as long as its average moisture in the cylinder declined to 14%. Then we took grain samples from each cylinder at a depth of 5–10 cm to determine mycological contamination.

To compare the results, the same experiments were carried out in a natural ventilation cylinder 9 with the

Table 1. **Mycological contamination of the grain used in the experiments**

Mycological contamination	Crops		
	wheat	malty barley	fodder barley
Number of extracted types of micromycetes	14	12	11
Dominant species of micromycetes	*Alternaria alternata* *Fusarium culmorum* *F. avenaceum* *F. tricinctum* *Penicillium chrysogenum*	*Aspergillus flavus* *Drechslera sorokiniana* *Fusarium culmorum* *F. tricinctum* *F. chlamydosporum*	*Alternaria alternata* *Fusarium culmorum* *F. tricinctum* *F. avenaceum*
Number of micromycetes spores cfu g^{-1}	$1.8 \times 10^3 \pm 390$	$5.5 \times 10^3 \pm 690$	$1.6 \times 10^4 \pm 1580$

speed of air filtration 0 m/s. The natural ventilation cylinder with grain was standing in the same room beside the drying jig. It was placed on 10 cm high grids so that the surrounding air could easily penetrate into the grain mound. During the experiment, the grain temperature in the natural ventilation cylinder was measured not in the upper layers but in the middle of the mound.

Petri dishes with Chapek's environment were used for the extraction of micromycetes. The dishes with grain were kept in a thermostat at a temperature of 26 ± 2 °C. Sprout mould fungi were purified and identified using the method of light microscopy according to descriptions of various authors. The quantitative grain contamination with micromycete propagules was estimated using the attenuation method. To distinguish the kinds of micromycetes and estimate the number of their propagules, three reiterations were conducted for each grain sample (Ellis, 1971; Ellis, 1976; Domsch et al., 1980; Gerlach, Nirenberg, 1982; Ramirez, 1982; Lugauskas et al., 1997; Lugauskas et al., 2002).

RESULTS AND DISCUSSION

After flailing the mycological contamination of grain increases. It depends on the soil and surrounding conditions, as well as on the cultivation, harvesting and transportation technologies (Bartels, Rodemann, 2003; Obst et al., 2000; Krasauskas et al., 2005). Therefore, in the storehouse the grain first of all is dried to prevent the development of mould fungi.

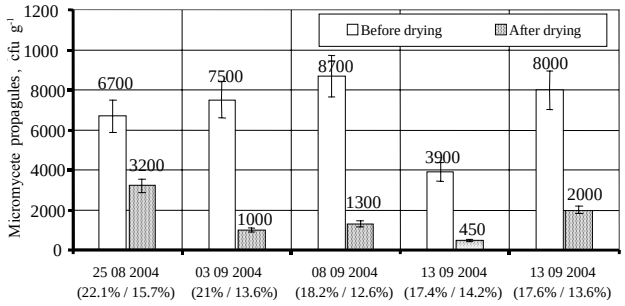

Fig. 2. The number of micromycetes in wheat dried in mixed-flow dryer (in brackets grain moisture before and after drying is shown)

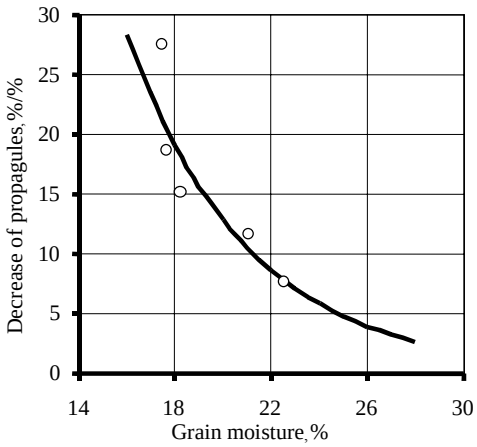

Fig 3. Reduction of micromycete propagules in drying grain

In the mixed-flow dryer, the grain mound, slowly scrolling down and blown over with the drying agent, is dried. The temperature of the drying agent depends on the grain, its initial moisture and the purpose. In our study, the grain was ventilated with the surrounding air heated up to 90 °C. The high temperature of the agent guarantees intense drying. The moisture of the dried wheat did not exceed 14.5%, except on 25 August (Fig. 2). On that day the highest initial moisture of grain (22.1 ± 0.076%) was fixed. However, after drying the wheat still showed a moisture level favourable for mould fungi (15.7 ± 0.068%).

The amount of micromycete propagules after drying declined 2.07 to 8.67 times. The smallest amount of mould fungi (from 6700 ± 840 to 3200 ± 280 cfu g^{-1}) perished on 25 August when the initial grain moisture was highest. On reducing the grain moisture by 1%, the number of mould fungi propagules on average declined by 7.68%. On the other hand, with declining the initial grain moisture this indicator increased (Fig. 3). On 13 September, when grain with 17.4 ± 0.091% of moisture was dried, on extracting 1% of moisture from wheat the propagules of micromycetes on average decreased even by 27.6%.

The dependence of the quantity of mould propagules on the initial moisture of grain was estimated; its coefficient was found to equal to 0.872:

$$D_i = \frac{674}{e^{0,1975 \cdot \omega_0}},$$

where D_i is the intensity of decrease of micromycete propagules, %/%; w_0 is the initial grain moisture, %.

The drier the grain, the more dangerous the drying process is to mould fungi, because in this case not only grain moisture decreases but also its temperature increases. At the beginning of drying, moisture intensively transpiring from the grain surface quenches the grain and does not allow it to heat, although the grain is washed with the hot drying agent. In addition, evaporation intensity slows down (Kröll, 1989; Kuzmienė et al., 1991). Only then the grain starts to heat. Its temperature starts to reach the agent's temperature. Since then the vitality of mould fungi is influenced by the grain moisture and its temperature. Temperature measurements of the grain effusing out of the drying zone showed that the grain in the flow dryer heated up to a temperature of 34.3 ± 3.5 °C which by 10–15 °C exceeds the optimal temperature of the development of mould fungi on grain.

On small and medium farms, grain is usually dried by active ventilation in a crop-bin. It is a cheap way of drying, because natural air drying qualities are made use of. It does not need any expensive and complicated equipment. Moreover, the same bin and ventilation

system can be employed for keeping grain during the storage period. However, some essential disadvantages are common to the active ventilation too. Here grain is simply dried in a stationary layer, and therefore the temperature of the dried agent is limited. It cannot exceed 35–45 °C. Grain is mostly dried with the surrounding air or air heated just by a few degrees. As a result, the process of drying is slow. Moreover, a ventilated grain layer dries unevenly. In the grain mound three zones emerge – those of dried, drying and humid grain. The drying process starts in the lower layer. Due to moisture evaporation from the grain, the agent cools down, moistens up to the balancing moisture and loses the drying qualities. Further leaking through the humid layers of the grain above, it does not absorb moisture any more and does not dry the grain. Only when in the lower layers the grain desiccates to the balancing moisture the temperature of the agent starts to rise, whereas in the upper layer it falls. It appears that the drying zone has expanded upwards. Thus it little by little expands in the direction of the air flow and involves the new layers of the humid grain. The drying is finished when the drying zone reaches and dries the upper layers of the grain mound. However, because of the slow and uneven drying process, favourable conditions for the development of micromycetes persist long.

The speed of the drying zone expansion depends on the parameters of the drying agent, the dried product and ventilation intensity. We have estimated that after some time since the beginning of drying, in the upper layers the drying agent temperature decreased by 75%. With such relative moisture, the grain balanced moisture will be approximately 14–15%. The point is reached when the moisture of the environment becomes too low for the development of mould fungi. For drying wheat, the temperature and relative humidity of surrounding air were 21.1 ± 2.0 °C and 68.5 ± 9.3% while for drying malty barley they were 19.7 ± 2.0 °C and 79.9 ± 12.2% and for drying fodder barley 19.0 ± 1.34 °C and 67.2 ± 7.8%. While ventilating wheat at the rate of 0.19 m/s, a 75% relative humidity in the upper layers of the dried wheat was reached after 39 h (Fig. 4a). On reducing the speed of air to 0.15 m/s the drying time prolonged up to 53 h, whereas ventilating at 0.10 m/s the drying time was 88 h, i.e. in the upper layers wheat was dried to 14–15% 27 h, 17 h and 10 h later than the whole mound. The relative air humidity in the upper malty barley mound decreased to 75% after 71 h (at v = 0.24 m/s), 184 h (at v = 0.11 m/s) and 228 h (at v = 0.09 m/s) (Fig. 4b), and when drying fodder barley after 63 h (at v = 0.18 m/s), 133 h (at v = 0.11 m/s) and 177 h (at v = 0.09 m/s) (Fig. 4c). Thus, grain moisture of 14–15% in the upper layer was reached 5 to 43 h later than in the mound on average.

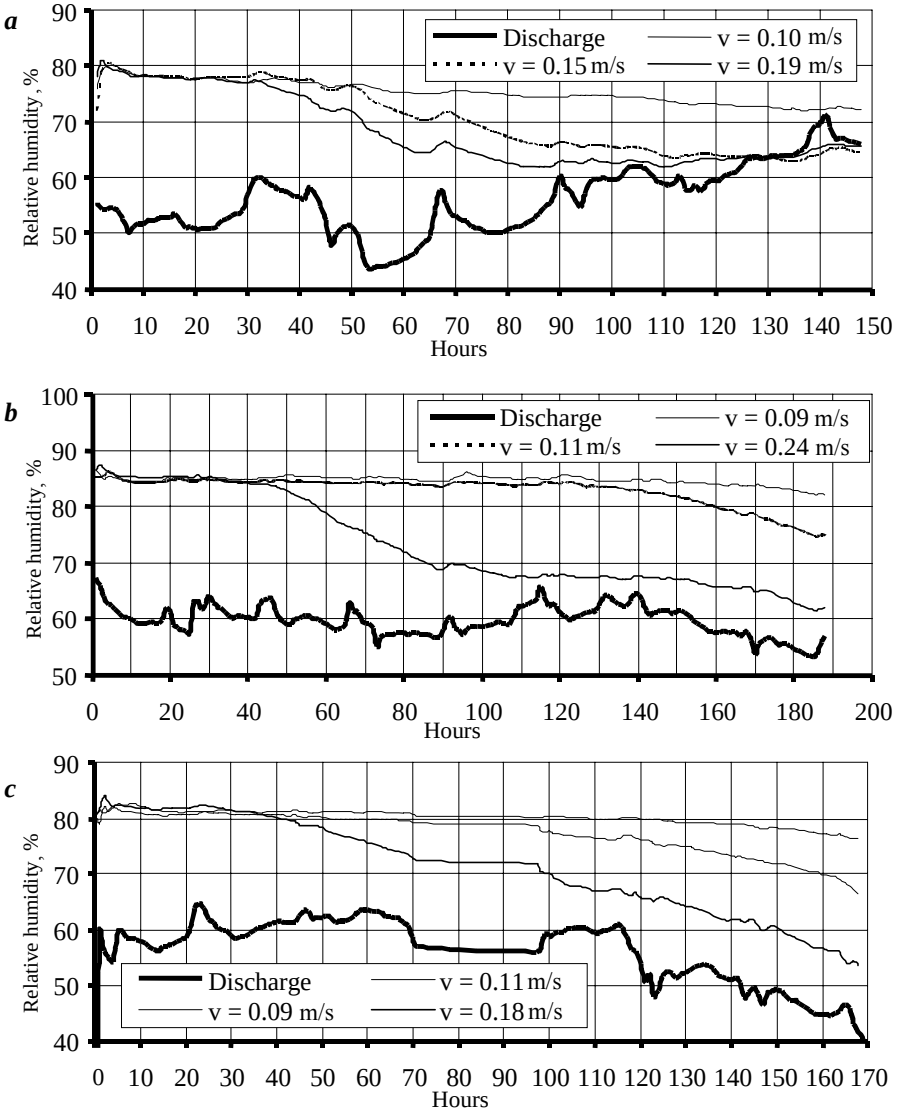

Fig. 4. Relative air humidity in the top layer of grain mound being dried: *a* – in wheat mound; *b* – in malty barley mound; *c* – in fodder barley mound

Air filtration suppressed the development of micromycetes (Fig. 5). Ventilating the grain with the air flow of 500 $m^3/(t \cdot h)$, by 48% less propagules were found than in under coercion non-ventilated grain. On the other hand, consequent ventilation intensity did not have a sizeable influence on the number of micromycetes in the grain.

We found a mathematical expression for the ventilation

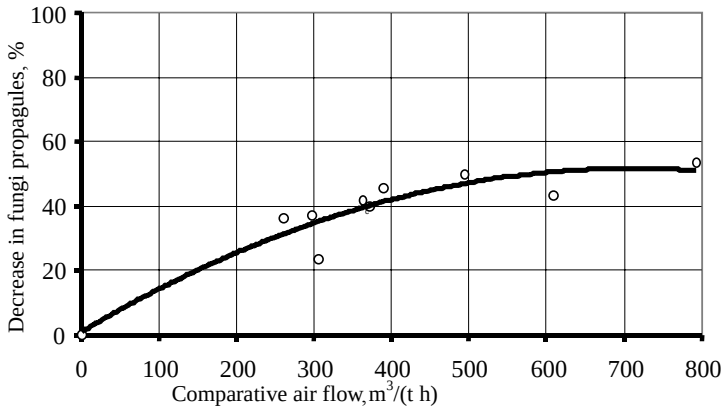

Fig. 5. Impact of ventilation intensity on the number of micromycetes in grain

intensity impact on the mould fungi propagule number; its determination coefficient is equal to 0.876:

$$N_{ksv} = -0{,}0001 \cdot Q^2 + 0.1435 \cdot Q + 0.7261,$$

where N_{ksv} is a decrease of micromycete propagules, %, and Q is the comparative air flow, $m^3/(t \cdot h)$.

After drying by active ventilation, the general number of the species of fungi decreased (Table 2). Field fungi, *Fusarium* and *Alternaria*, started to dominate in the grain. Species of the genera *Penicillium* and *Aspergillus* decreased, because their conidia are

Table 2. **Mycological contamination of grain after the drying research**

Wheat				
Mycological contamination	Air filtration velocity			
	v = 0 m/s	v = 0,10 m/s	v = 0,15 m/s	v = 0,19 m/s
Number of extracted types of micromycetes	7	9	10	10
Dominant species of micromycetes	*Alternaria alternata* *Fusarium culmorum* *Penicillium* spp.	*Alternaria alternata* *Fusarium culmorum*	*Alternaria alternata* *Fusarium culmorum* *Penicillium* spp.	*Alternaria alternata* *Fusarium culmorum* *F. avenaceum*
Number of spores of micromycetes cfu g^{-1}	$2.2 \cdot 10^3 \pm 320$	$1.4 \cdot 10^3 \pm 300$	$1.2 \cdot 10^3 \pm 180$	$1.1 \cdot 10^3 \pm 160$
Malty barley				
Mycological contamination	Air filtration velocity			
	v = 0 m/s	v = 0.09 m/s	v = 0.11 m/s	v = 0.24 m/s
Number of extracted types of micromycetes	8	10	9	11
Dominant species of micromycetes	*Bipolaris sorokiniana* *Fusarium culmorum* *Aspergillus flavus*	*Bipolaris sorokiniana* *Fusarium chlamydosporum* *F. tricinctum* *F. avenaceum*	*Bipolaris sorokiniana* *Fusarium tricinctum* *F. culmorum*	*Bipolaris sorokiniana* *Fusarium tricinctum* *F. culmorum*
Number of spores of micromycetes cfu g^{-1}	$4.3 \cdot 10^4 \pm 6100$	$2.7 \cdot 10^4 \pm 4900$	$2.5 \cdot 10^4 \pm 3100$	$2.0 \cdot 10^4 \pm 2800$
Fodder barley				
Mycological contamination	Air filtration velocity			
	v = 0 m/s	v = 0.09 m/s	v = 0.11 m/s	v = 0.18 m/s
Number of extracted types of micromycetes	10	11	12	11
Dominant species of micromycetes	*Fusarium culmorum* *F. avenaceum*	*Fusarium culmorum* *F. avenaceum* *F. tricinctum*	*Alternaria alternata* *Fusarium culmorum* *F. avenaceum* *F. tricinctum*	*Fusarium culmorum* *F. avenaceum* *F. tricinctum*
Number of spores of micromycetes cfu g^{-1}	$3 \cdot 10^4 \pm 4100$	$2.3 \cdot 10^4 \pm 3600$	$1.8 \cdot 10^4 \pm 2000$	$1.7 \cdot 10^4 \pm 1600$

smaller and more vulnerable to unfavourable conditions.

However, employment of active ventilation does not mean that there will be less micromycetes and their propagules after drying than before it. A correct control of the drying process is very important. The most frequent mistake is the drying of grain in unvafourable environmental conditions when the ventilated grain is not only dried but also humidified. Therefore, during all period of drying (188 h), from malty barley, at the rate of 0.24 m/s air filtration, 2.21 kg water was evaporated whereas 1.01 kg (45.7% of the extracted moisture) was absorbed. In other cases the absorbed moisture made up 46.6% (at v = 0.11 m/s) and 56% (at v = 0.09 m/s), whereas under coercion non-ventilated grain absorbed even 67% of vaporized water. It prolongs the process of drying and the favourable conditions for the development of mould fungi in the grain mound. Because of the incorrect control of the drying process, the number of micromycete propagules in the grain declined only in wheat. In fodder barley, the propagule number increased insignificantly by 6.25% (at v = 0.18 m/s), 12.5% (at v = 0.11 m/s) and 43.8% (at v = 0.09 m/s). The quality of malty barley suffered most. Micromycetes increased in them 3.64 to 4.9 times, because the drying of malty barney lasted longest.

CONCLUSIONS

1. Grain drying is an effective means to slow down the development of micromycetes – potential mycotoxin producents.

2. In a mixed-flow dryer, in grain dryed at a temperature of 90 °C, the number of micromycete propagules decreases 2.07 to 8.67 times. The intensity of decrease under drying is conversely proportional to the initial grain moisture.

3. Drying grain by active ventilation, favourable conditions for micromycete development remain longest in the upper layers of the mound where by the end of drying air humidity exceeds 75%.

4. Air filtration through the humid grain layer suppressed the development of mould fungi, but a comparative ventilation intensity increase of more than 500–600 $m^3/(t \cdot h)$ does not have any impact on the amount of micromycetes.

5. Grain drying by active ventilation in unfavourable environmental conditions slows down the development of micromycetes but does not protect grain from mycological contamination increase.

Received 20 May 2006
Accepted 4 August 2006

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MIKOTOKSINŲ PRODUCENTAI GRŪDŲ SLUOKSNYJE

S a n t r a u k a

Mikromicetai ne tik kenkia grūdų kokybei, bet ir yra potencialūs mikotoksinų producentai. Patikimiausias būdas neužteršti grūdų mikotoksinais – išvengti jų atsiradimo. Todėl, mažinant maisto produktų užkrėstumą mikotoksinais, labai svarbios prevencinės priemonės, nukreiptos prieš mikromicetus. Mažas drėgmės kiekis grūduose yra svarbiausias veiksnys, silpninantis mikromicetų gyvybingumą. Labai svarbu teisingai parinkti džiovinimo režimus ir išdžiovinti grūdus, kol mikromicetai jų nesugadino. Atliktais tyrimais buvo analizuotas kviečių, salyklinių ir pašarinių miežių džiovinimas šachtinėje džiovykloje ir aktyviosios ventiliacijos aruode, taip pat įvertinta šių džiovinimo technologijų įtaka grūdų mikologinei taršai.

Raktažodžiai: grūdai, džiovinimas, mikromicetai