

Investigation of physical prevention means to reduce mycological contamination of grain surface

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The effect of ozone (O₃) on the mycological pollution of ventilated grain surface and its drying process was studied. Investigations have revealed that with air–ozone mixture blown through grain mound, ozone splits while contacting the grain surface and disinfecting it, and this process can be evaluated by the ozone absorption factor (absorptivity). Ozone splitting and absorption depend on grain moisture; in columns of grain with a greater moisture content these processes are more intense. The effectiveness of grain disinfection depends on grain column height, moisture content, ozone concentration and the duration of ozone exposure. The influence of ventilation duration and ozonation on pollution was evaluated by changes of grain surface infection with micromycete propagules (cfu·g⁻¹). Ozone–air mixture used for active ventilation drying of grain (with ozone concentration of 700 ppb) enables reduction of drying duration by about 20% and of mycological pollution depending on moisture content *w* from 2.2 times (for grain *w* = 15.2%) to three times (grain *w* = 22.0%).

Key words: grain, moisture content, micromycetes, ventilation, ozonation

INTRODUCTION

The annual total wheat grain yield in the world reaches 628 million tons, one third of it being harvested in Europe. In Lithuania it amounts to 1.48 million tons (FAO Statistical Data, 2005). Losses caused by activity of microorganisms including mould fungi, bacteria and others reach about 2% of grain dry material (Нуриев, Рудобашта, 2001) and in some countries even up to 50% of the total yield (Jay, 1999). Mould fungi degrade not only the quantitative but also qualitative grain and grain product indices (Бутко и др., 2005; Young et al., 2006). In addition, the majority of mould fungi are able to synthesize in certain conditions mycotoxins harmful to humans and livestock (Lugauskas et al., 2002). The main mold fungi types producing mycotoxins are *Aspergillus*, *Fusarium* and *Penicillium* (Chulze et al., 1996). Their spread and mycotoxin production depend on many factors including agricultural technology of growing, temperature and moisture, conditions during crop harvesting and storage (Bartels, Rodemann, 2003; Marin et al., 1998; Baliukonienė, Bakutis, 2002; Homdork et al., 2000). Microorganism growth can be suppressed by drying grain in drying devices or by active ventilation, when moisture content in them is reduced to 13–14% (Zvicevičius et al., 2005). However, these measures do not ensure a complete extermination of microorganisms in grain (Глуценко и др., 2003). Disinfection of grain is a widely applied measu-

re to conserve its quality. As a means of grain disinfection (against mould fungi and their toxins) various chemicals were investigated (sodium hypochlorite, various antioxidants and others) (Andrews et al., 1997; Ramakrishna et al., 1991; Nesci et al., 2003). However, they are costly, inefficient and not ecological. More often grain is disinfected using phosphate hydrogen (PH₃) and methyl bromide (CH₃Br) (Kells et al., 2001), however, their application becomes more and more restricted in Europe as is already completely banned in the US (Allen et al., 2003). Reaction of methyl bromide with water releases methane gas, which is poisonous. CH₃Br reduces ozone and destroys its layer in the upper zone of the atmosphere (Kells et al., 2001). In addition, some microorganisms in stored grain become resistant to the above mentioned chemicals (Zettler, Cuperus, 1990). Scientists acknowledge that usage of chemical means causes a series of ecological, social and power engineering problems (Цугленок и др., 2003; Ryden et al., 2003).

Biological substances as a means of extermination of mould fungi micromycetes were also investigated (propion ferment, modified manano-oligosaccharides, agents obtained from bacteria *Erwinia herbicola* and others) (Цугленок и др., 2003), but their utilization technology is long and complex. In the US, attempts were made to inactivate mould fungi on barley grain surface using ether oil, but it reduces grain germinating ability (Paster et al., 1995). Physical disinfection

means are among the most promising ones being the least harmful to the environment, and they include grain cleaning, thermal processing, exposure to high frequency electromagnetic oscillation, to electron flux, luminescence or ozone and others (Цугленок и др., 2003; Cutrubinis et al., 2005; Ткачев и др., 2002; Голубкович и др., 2002; Закладной и др., 2003; Ramakrishna et al., 1991; Baba et al., 2004; Andrews et al., 1997). However, most of these technologies are very costly while others are not investigated sufficiently. With the issues of healthy nourishment and environmental ecology becoming more vital nowadays, the search for new and more environment-friendly grain disinfection means is urgent. Ozone (O_3), being a powerful oxidant (Лунин и др., 1998; Разумовский и др., 1983), can be utilized to oxidize many chemical compounds and microorganisms (Kim et al., 1999). Ozone is more acceptable ecologically than chemical disinfection means and when used leaves important grain quality properties unaffected (Mendez et al., 2003; Глуценко и др., 2003). The main advantage of this disinfection procedure is absence of harmful reaction products after exposure of microorganisms to it (Kim et al., 2003; Young et al., 2006). The ozone molecule remains in air until splitting into oxygen for 20 to 50 minutes, while in water it remains 1 to 10 minutes (Mason et al., 1997). Ozone has been used long enough for water disinfection (Kim et al., 2003) and for reduction of air microbiological pollution in buildings (Сторчевой, 2003). In addition, ozone is acting not only as an insecticide, but time also as a fungicide (Kells et al., 2001; Kliperis et al., 2005). Investigations of ozone influence on barley mould fungi revealed a positive effect on grain mycological cleanliness without lessening grain germinating power. Micromycetes of mould fungi are less resistant than spores to ozone exposure (Allen et al., 2003). Activity of mould fungi *Aspergillus flavus* can be inhibited by grain exposure to 5 ppm ozone concentration (Mason et al., 1997). Ozone was used also for detoxification of food products infected by mycotoxins (Маeba et al., 1988; McKenzie et al., 1997). In addition, ozone has been found to support the grain drying process, reducing its duration by about 25% (Тарушкин и др., 2001; Голубкович и др., 2002; Троцкая, Литвинчук, 2001). Small ozone concentrations ($20\text{--}30 \mu\text{g}\cdot\text{m}^{-3}$) positively influence plants and animals. However, ozone concentration above $180 \mu\text{g}\cdot\text{m}^{-3}$ is already dangerous for human health (Ryden et al., 2003). Therefore, utilization of ozone for grain disinfection must be investigated taking into account its initial concentration, exposure to ozone duration and efficiency, grain mound height and moisture content and flowing out ozone concentration. One of the most important factors is the intensity of ozone dissipation (absorption) in the grain layer.

The aim of the present work was to determine the intensity of grain exposure to ozone and its parameters,

ensuring a safe use of ozone as a physical prevention means to reduce the mycological pollution of grain surface at various moisture content values.

METHODS

Investigations of the efficiency of physical means (ozone) of reducing mycological pollution of grain surface were carried out in 2004–2005 at the Laboratory of Heat and Biotechnological Engineering Department of The Lithuanian University of Agriculture.

The test rig (Fig. 1) consisted of a centripetal air blower 1, chamber of constant static pressure 2 which was connected to cylinders 5. Each cylinder, 0.18 m in diameter and of 1.2 m in height, contained 22.0 kg of wheat grain. The parameters of air supplied for grain drying and ventilation were the same for each cylinder. The same ($0.12 \pm 0.02 \text{ m}\cdot\text{s}^{-1}$) velocity of air seepage through grain layer was chosen for each cylinder by regulating the position of the constrictors 4.

The mass value of grain was registered every four hours by weighing cylinders 5. The air temperature and relative humidity were measured using the ALMEMO sensors FH A646–21 (temperature data error $\pm 0.1 \text{ }^\circ\text{C}$, relative humidity error $\pm 2\%$). Measurement results were sent every 10 min to the ALMEMO 3290 data accumulating device.

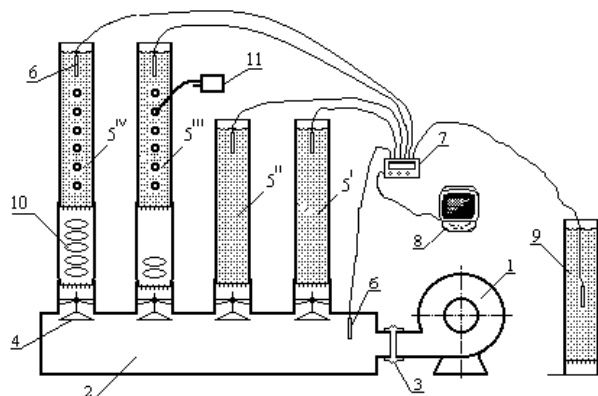


Fig. 1. Schematic diagram of the drying test rig:

1 – air blower, 2 – chamber of constant static pressure, 3 – flexible joint, 4 – restrictor, 5^I, 5^{II}, 5^{III}, 5^{IV} – ventilated cylinders with grain, 6 – sensors of temperature and moisture content, 7 – secondary ALMEMO measurement unit, 8 – computer, 9 – natural ventilation cylinder with grain, 10 – ozonators, 11 – measurement unit of ozone concentration

Investigation of ozone influence on the grain drying process. To determine the effect of ozone exposure on grain drying intensity, two ozonators were mounted at the bottom of the third cylinder and five ozonators 10 at the bottom of the fourth cylinder. At an established seepage velocity ($0.12 \text{ m}\cdot\text{s}^{-1}$), each ozonator produced ozone concentration in the air supplied for drying, amounting to $140 \pm 5 \text{ ppb}$. Test rig cylinders were filled with winter wheat ‘Taurus’ grain with the initial moisture content $23.2 \pm 0.3\%$. The wheat was harvested on 16.08.2005, and the same day its drying was

commenced in the rig of active ventilation. Grain was ventilated for 8 days 8 hours daily (from 11.00 to 19.00). In the rest of the daytime the test rig was inoperative and grain was not supplied to the cylinders because of an increased humidity of the surrounding air. Grain was ventilated until its average moisture content in a cylinder diminished to 14%.

The natural ventilation cylinder 9, placed near the test rig, was filled with the control sample of wheat grain. The cylinder was mounted on a 10 cm high grating to allow the surrounding air to flow freely into the grain column. However, grain mound height in it was less than in the actively ventilated cylinders, reaching 0.8 m. The temperature was measured in the middle part of the grain mound in this cylinder.

Investigation of ozone permeability in the mound of grain exposed to ozone. Grain of wheat 'Taurus' with different moisture content was poured into the cylinders of the experimental rig: into the first 5^I and the fourth 5^{IV} – $w = 22.0 \pm 0.4\%$, while into the second 5^{II} and the third 5^{III} – $w = 15.2 \pm 0.1\%$. Five ozonators 10 were mounted at the bottom of the third 5^{III} and the fourth 5^{IV} cylinders. Grain was ventilated five hours daily for five days (from 12.00 to 5 p. m.). During the rest of the daytime the rig was switched off. Ozone concentration in the grain mound (in cylinders 3 and 4) was recorded every 15 min using an 11 measurement device (AHLBORN Ozon-Sonde FY A600-03). Ozone penetration rate and absorptivity were established from its variation. The initial number of micromycete propagules for 22.0% moisture grain was $M_0 = 1.1 \times 10^4 \pm 1.5 \times 10^3$ cfu·g⁻¹, while for 15.2% $M_0 = 7.1 \times 10^3 \pm 1.3 \times 10^3$ cfu·g⁻¹.

The effects of the exposure to ozone and ventilation duration were evaluated according to the number of colony forming units (cfu) of micromycetes. Every day grain samples were taken from the upper and bottom parts of each cylinder for determination of their ecological pollution.

Petri dishes with Chapek media were used for micromycete release. Petri dishes with grain were kept in a thermostat at a temperature of 26 ± 2 °C. Fungi were separated, purified up to monocultures and later identified by light microscopy, taking into account their physiological and morphological features. For identification, methodologies of previous researcher's were used (Domsch et al., 1980; Nelson et al., 1983; Lugauskas et al., 2002). Quantitative pollution of grain by micromycete propagules was established using the dilution method. For establishing the species of micromycetes and the number of their propagules, for each grain sample the procedure was repeated three times.

The obtained data were evaluated using the methods of dispersion and correlation (regressive analysis).

RESULTS AND DISCUSSION

Grain mound permeability to ozone. Ozone in the grain layer moves because of diffusion, i. e. its mole-

cules move through pores from points with a higher concentration to points with a lower concentration along the concentration gradient until the distribution balance is reached.

Then penetration of ozone may be expressed by the kinetic-diffusion equation:

$$\frac{\partial C}{\partial t} = D \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C}{\partial r} \right) + \frac{\partial^2 C}{\partial h^2} \right] - v_f \frac{\partial C}{\partial h} - kC, \quad (1)$$

where C – ozone concentration; D – diffusion; r – radius of the grain mound bottom; h – grain mound height; k – factor of ozone absorption; v_f – air seepage velocity in the grain layer; t – duration of exposure to ozone.

The member in the square brackets evaluates diffusion of ozone molecules in transversal direction while the second member evaluates diffusion in longitudinal direction. The second member on the right side of equation (1) evaluates ozone penetration because of its velocity, and the last member on the right side of this equation evaluates ozone absorption by grain surface. If ozone moves into the grain layer together with air supplied by the ventilator, i. e. at a certain velocity and pressure, in equation (1) the member representing diffusion of ozone molecules may be neglected. Then the general kinetic equation for ozone penetration in a grain mound is

$$\frac{dC}{dt} = -kC. \quad (2)$$

When the grain mound cross-section area is S , the ozone absorption rate:

$$\frac{dC}{dt} = -k C S. \quad (3)$$

Ozone concentration variation in time or penetration rate v , with evaluation of ozone absorption in the grain layer, may be expressed as follows

$$v = \frac{dC}{dt} = \frac{v_f}{V} S (C_0 - C) - k C S, \quad (4)$$

where V – grain mound volume; C_0 – ozone concentration in air supplied to grain layer.

The first member on the right side of the equation (4) evaluates the variation of concentration in time in case of absence of absorption (4), while the second member shows the absorption of ozone in reaction with the grain surface.

From equation (4), the ozone absorption factor can be expressed as

$$k = \frac{\frac{v_f}{V} S (C_0 - C) - v}{C S}. \quad (5)$$

The magnitude of the ozone absorption factor (absorptivity) k can be determined in two ways: by substituting in equation (4) experimentally obtained ozone concentration values in separate moments of time or by solving equation (4) by iterations.

In the case under consideration, the numerical value of the k factor was determined experimentally. Ozone concentration was registered periodically every 15 min (Figs. 2 and 3) and the penetration rate ν was calculated (Fig. 4). Substitution of these data into equation 5 allows determining the absorption factor k (absorptivity); its values are shown in a diagram (Fig. 5).

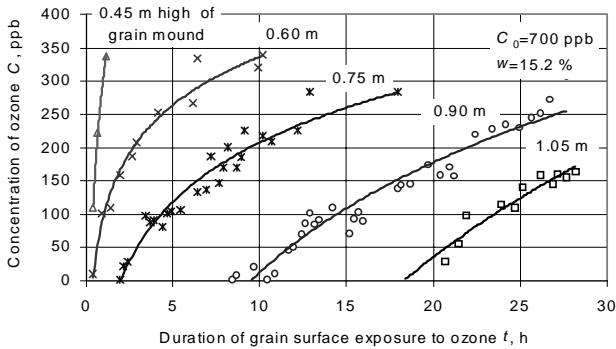


Fig. 2. Variation of ozone concentration C at the h height of the grain mound as a function of exposure to ozone duration t at the initial number of micromycete propagules $M_0 = 7.1 \times 10^3$ cfu·g⁻¹

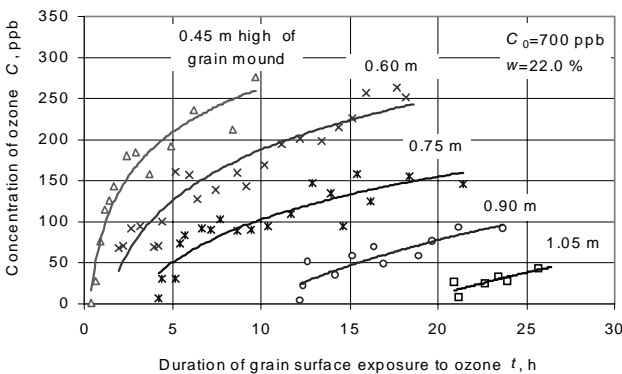


Fig. 3. Variation of ozone concentration C at the h height of the grain mound as a function of exposure to ozone duration t at the initial number of micromycete propagules $M_0 = 1.1 \times 10^4$ cfu·g⁻¹

Experimental investigations confirmed conclusions of researchers (Ксенз, 2003; Kells et al., 2001; Троцкая, Литвинчук, 2001, etc.) that ozone penetration is influenced mainly by the length of exposure to ozone (Figs. 2 and 3).

In addition, ozone penetration depends also on the properties of ozonated products (Kim et al., 1999). With a longer time of exposure to ozone its presence was registered each time in a higher layer of the mound. The variation character of ozone concentration depended on grain mound (column) height. In the beginning of the process, ozone was penetrating (up to 0.60 m) the bottom grain layers very rapidly, later its concentration increased at a lower rate (Fig. 2). At mound heights over 0.75 m, ozone concentration grew less intensively and more gradually. The character of ozone concentration variation in the mound with a greater grain moisture

($w = 22.0\%$) (Fig. 3) resembled the variation for grain with $w = 15.2\%$ moisture (Fig. 2). However, ozone at the same mound height was registered in case of greater moisture later if compared with drier grain. In grain with a 15.2% moisture, ozone at the height of 0.60 m was registered already after 0.5 h, and at 0.75 m 2.5 h (Fig. 2), in grain with a 22.0% moisture it was registered after 2 h and 4.5 h respectively (Fig. 3). The difference was still greater (about 4 h) in the upper layers of the mound (0.90 m and 1.05 m).

During exposure of the grain surface to ozone its absorption was observed (Закладной и др., 2003). Mendez et al. (2003) noticed that the ozone penetration rate ν in a grain mound was not constant. Our investigations indicate that it is directly proportional to the duration t of exposure to ozone and depends on the grain mound height coordinate h . After analysis of ozone penetration (concentration) measurement data, which were measured every 15 min during the whole period, the ozone penetration rate ν was established and its variation diagram was drawn (Fig. 4). The rate ν each next day (every 5 h) at the same grain mound height was greater. After two days (10 hours of ozonating) at a height of 0.60 m the ozone penetration rate reached 5.04 ppb·min⁻¹, while after 3 days (15 h) it reached 8.10 ppb·min⁻¹ (Fig. 4). In the upper layers of the grain mound the ozone penetration rate was not only less, but also it was increasing slower (at 0.75 m after 10 h it reached the value of 1.19 ppb·min⁻¹). The data presented in Fig. 4 suggest that in ozonated grain the ozone penetration and absorption rates are greater while absorption is less.

Ozone absorption in a grain layer was found to depend on ozone concentration in the air supplied to the layer and on the duration of ozone exposure. (Троцкая, Литвинчук, 1998; Ксенз, 2003; Закладной и др., 2003), velocity of air supplied (Kells et al., 2001; Mendez et al., 2003) and on temperature (Allen et al., 2003). In addition, Allen et al. (2003) state, that the intensity

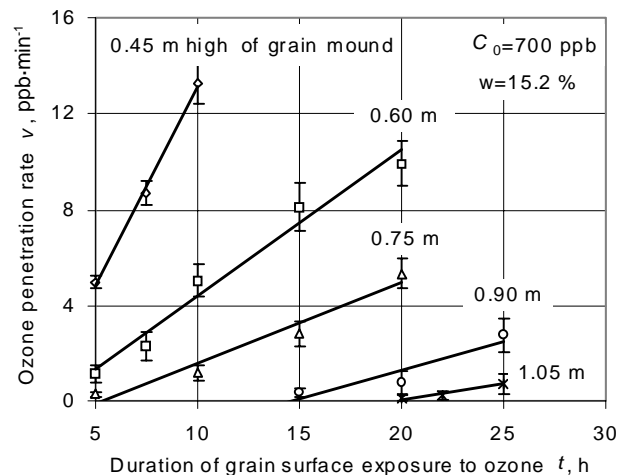


Fig. 4. Dependence of ozone penetration rate ν on grain surface exposure to ozone duration t at the initial number of micromycete propagules number $M_0 = 7.1 \times 10^3$ cfu·g⁻¹

of ozone absorption indicates pollution of grain surface. These authors underline that the greater mycological pollution the slower ozone penetration into the grain medium.

By substituting the obtained values of ozone penetration rates ν into equation (5) the ozone absorption factor k was calculated (Fig. 5). Experimental investigations carried out in 2005 have shown that ozone absorption in the grain layer is influenced not only by the parameters mentioned above, but also by the grain moisture w and mound height h (in the upper layers of the mound absorption is greater). As the mound of ventilated grain begins to dry from the bottom layers (Zvicevičius et al., 2005), and with grain drying and moisture content diminishing the numbers of microorganisms also diminish, ozone absorption in the upper layers increases.

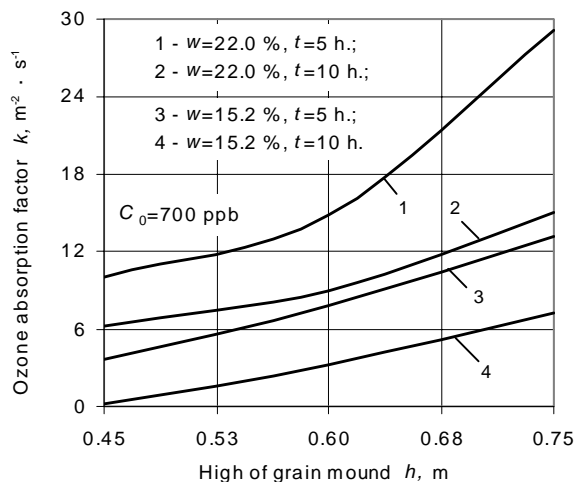


Fig. 5. Influence of grain moisture w , height of mound (column) h and exposure to ozone duration t on the absorption factor k of ozone

We have found that ozone in reaction with a moister grain surface whose mycological pollution is usually higher and with microflora present on it splits more rapidly. As ozone is a strong oxidizer, at the same it destroys microorganisms on the grain surface, including mould fungi. Thus, in case of moister grain, ozone penetrates its layer slower with a longer reaction with the grain surface and its microorganisms, so its destruction is more effective because of a longer exposure.

Grain drying by active ventilation is a cheap and rather universal drying procedure, which enables reduction of harmful effects by microorganisms (Zvicevičius et al., 2005). The number of micromycete propagules in not ventilated grain grew insignificantly during the first two days (reaching 20%), however, after 5 days it became nearly doubled (1.9×10^4 cfu·g⁻¹) as compared with the initial value (1.1×10^4 cfu·g⁻¹).

While drying grain by active ventilation using air with ozone, it is possible not only to inhibit the development of mould fungi but also to reduce their amount. The mycological pollution of grain surface was reduced

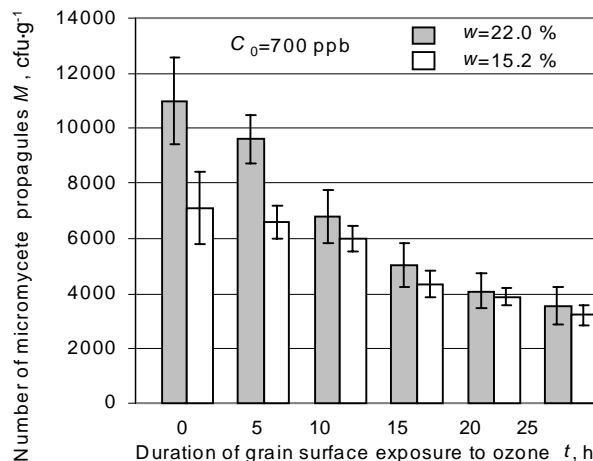


Fig. 6. Influence of wheat grain moisture w and exposure to ozone duration t on mycological pollution of grain surface

more significantly while ozonating the grain mound with a greater moisture content (Fig. 6) and ozone absorption. The mycological pollution of moister (22.0%) grain during exposure to ozone for 25 h was reduced about three times, while of less moist (15.2%) grain 2.2 times. During active ventilation, when ozone content in the air was 700 ppb, the number of micromycetes for 22.0% moisture grain diminished after 5 days to 3.5×10^3 cfu·g⁻¹, while for 15.2% moisture grain to 3.2×10^3 cfu·g⁻¹, in agreement with the reports that ozone in media with a greater surface moisture content is a more active oxidizer (Kim et al., 2003; Allen et al., 2003). With a 280 ppb ozone concentration in the drying agent, the change of grain mycological pollution as compared with ventilation without ozone in the air was not statistically reliable.

Our results imply that an efficient use of ozone is possible only in cases of columns (mounds) of sufficiently moist grain ($w > 18\%$). Therefore, further investigations should include determination of moisture limits with the aim to ensure the feasibility of ozone application.

Investigation of ozone influence on grain drying process. Ozone used together with a drying agent intensifies the grain drying process, particularly during the first three days (Fig. 7).

In case of a drying agent without ozone, the grain moisture diminished from 23.2% to 22.1% (Fig. 7, curve 1), while with 700 ppb ozone concentration in the air it changed from 23.2% to 19.0% (Fig. 7, curve 3). Later the grain drying intensity differed only slightly; however, grain moisture of 14% was reached two days earlier than in the case of air without ozone. The drying agent with the ozone concentration 280 ppb had little influence on the drying process. A more intensive reduction of grain moisture with grain surface exposed to ozone is possible because of ozone interaction with water, i. e. moisture content of exposed material causes changes in the physical, chemical and thermal proper-

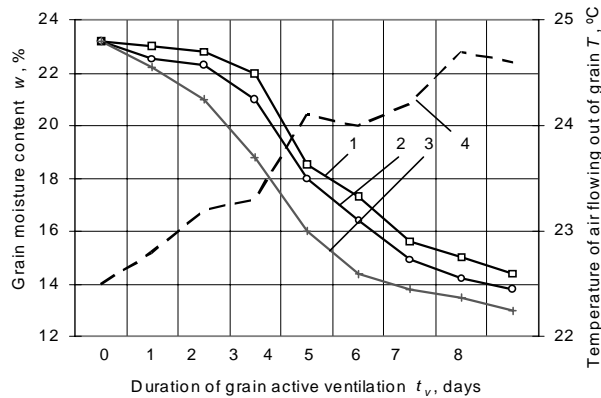


Fig. 7. Influence of wheat grain 'Taurus' ventilation duration t_v on variation of grain moisture w : 1 – moisture of grain ventilated without ozone; 2 – moisture of grain ventilated when ozone concentration was 280 ppb; 3 – moisture of grain ventilated when ozone concentration was 700 ppb; 4 – average temperature of air escaping from grain

ties of water (Глуценко и др., 2003). In addition, the drying process is considerably influenced by the temperature of the drying agent reaching 24 ± 1.2 °C. During the drying process, the temperature of air escaping from the grain mound became almost equal to the surrounding air temperature (Fig. 7).

CONCLUSIONS

1. When the air–ozone mixture is blown through the grain mound, ozone splits in contact with the grain surface and disinfects it; this process is evaluated by the ozone absorption factor (absorptivity). An appreciable ozone concentration flow spreads in the direction of the drying agent movement until it reaches the upper layers of the grain mound. A direct link exists between grain moisture and ozone absorption, and moister grain has a greater absorption capacity.

2. The efficiency of disinfection by ozone and its positive influence on grain drying depend on ozone concentration in the air stream blown, the length of exposure to ozone, mound height and the ozone absorption factor, which diminishes when the time of exposure to ozone increases.

3. The ozone–air mixture used for active ventilation drying of grain (with ozone concentration 700 ppb) enables reducing the duration of drying by about 20% and of mycological pollution, depending on moisture content w , from 2.2 (grain $w = 15.2\%$) to three times (grain $w = 22.0\%$).

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**FIZIKINIŲ PREVENCIJOS PRIEMONIŲ GRŪDŲ
PAVIRŠIAUS MIKOLOGINEI TARŠAI MAŽINTI
TYRIMAI**

S a n t r a u k a

Tirtas ozono (O_3) poveikis ventiliuojamų grūdų paviršiaus mikologinei taršai ir jų džiūvimo procesui. Tyrimais nustatyta, kad kontaktuodamas su grūdų paviršiumi ir jį dezinfekuodamas ozonas skyla, o tai apibūdinama ozono sugerties koeficientu.

Ozono skilimas ir sugertis drėgnesnių grūdų sampile yra intensyvesnis. Grūdų dezinfekavimo efektyvumas priklauso nuo grūdų sampilo aukščio, drėgnio, ozono koncentracijos ir ozonavimo trukmės. Ventiliavimo trukmės ir ozonavimo įtaka vertinta pagal grūdų užkrato mikromicetų pradais ($ksv \cdot g^{-1}$) pokyčius grūdų paviršiuje. Džiovinant grūdus aktyviaja ventilacija su ozono ir oro mišiniu ir naudojant 700 ppb O_3 koncentraciją, džiovinimo trukmę galima sumažinti apie 20%, o mikologinę taršą, atsižvelgus į grūdų drėgnį w , nuo 2,2 ($w = 15,2\%$) iki 3 ($w = 22,0\%$) kartų.

Raktažodžiai: grūdai, drėgnis, mikromicetai, ventilacija, ozonavimas