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APPLICATION OF OZONE FOR REDUCTION OF MYCOLOGICAL INFECTION IN WHEAT GRAIN

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Abstract: In 2004-2005 means were sought to clean grain from microbiological contamination during transportation and storage. For this purpose, grains with a moisture content of 23.2% of the "Tauras" variety were selected and ventilated daily for 8 hours until grain wetness was reduced to 14.0%. The effect of ventilation duration and ozone impact was evaluated according to the changes in grain contamination with micromycetes propagules (cfu·g^{-1}), and alternation of micromycetes species on the grain surface. At drying grains by active ventilation with an ozone – air mixture, at O_3 concentration of 700 ppb, the drying period was reduced by about 20%, and mycological contamination depends on initial grain moisture content (w): when w=15.2%, contamination was reduced by up to 2.2 times, and when w=22.0% – up to 3 times. At the same time, the composition of micromycetes species on the grain surface changed significantly: in non-ventilated grain there were detected micromycetes of 26 species, and in ventilated grain – of 11 species. Efficient ozone impact was established only when the mound of wet (w>18.0%) grains was exposed to ozone.

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INTRODUCTION

One of the most important factors determining food quality is mycological contamination of plant raw material which starts at the growing stage of grain and seeds, proceeds during production and marketing of products, and terminates after the product is consumed. Plants in all their stages have close contacts with living and non-living bodies of the environment. Therefore they contain plenty of microorganisms on their surface and inside that deteriorate the products' nutritive value and are dangerous to human and animal health. According to FAO statistics, annual wheat production is around 628 million tons. One third is produced in Europe. In Lithuania, wheat production reaches 1.48 million tons (FAO, 2005). Because of harmful microorganisms: bacteria, microscopic fungi, etc. the waste of grain dry matter is around 2% [34], and in some countries even up to 50% of all harvests [17]. Microscopic fungi deteriorate not only quantitative but also qualitative parameters of grains as well as their products [6, 16].

Researchers in many countries [8, 12, 23, 26] indicate the danger raised by micromycetes during their development when they release toxic secondary metabolites, in this case on grain surface that can cause functional, cancerous and psychological disorders of human health. It

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was established that mycotoxins could be produced by various micromycetes species developing under Lithuanian climatic conditions on wheat and other cereals grown for human food. Meanwhile, storage grain is mostly contaminated with micromycetes of the following genera: *Alternaria*, *Fusarium*, *Aspergillus*, *Eurotium*, *Penicillium*, *Cladosporium*, *Bipolaris*, *Phoma*, *Myrothecium*, *Acremonium*. The composition and prevalence degree of micromycetes species depend on plant species, habitat, soil type, agrotechniques, and meteorological conditions during vegetation, especially during grain ripening and harvest, as well as the method of harvesting and storage conditions [4, 7, 15, 25].

Microorganisms' development can be limited or inhibited by drying grains in dryers or by active ventilation reducing moisture content until 13÷14% [47]. However, the means mentioned do not ensure total destruction of microorganisms in grains [13]. Therefore disinfection by various chemicals is widely used to preserve grain quality. In order to limit the negative effect of micromycetes and the released toxic secondary metabolites on grains the following preparations were used: sodium hypochlorite, various antioxidants, etc. [2, 33, 36]. On the other hand, the mentioned chemicals are expensive and environmentally dangerous. Most often, grain was disinfected by phosphorus hydrogen (PH_3) and methyl bromide (CH_3Br) [18], but limitations for applying the latter in Europe are increasing, and in America it is totally banned [1]. The cause of the ban was the toxic methane compound released as the reaction of methyl bromide with water. Methyl bromide dissociates ozone and depletes its layer in the stratosphere [18]. In addition, some insects infesting stored grain became resistant to the aforementioned chemical preparations [46]. Scientists acknowledge that the application of chemical means raised many ecological, sanitary, social, health protection and energy problems [9, 38].

In addition, the following biological compounds were used to control micromycetes: propane yeast, modified mannanoligosaccharides, *Erwinia herbicola* bacteria preparations, etc. [9]. The technology of biological preparations application is complex, it takes a long time to prepare them, and the expected effect is not always obtained. In America, there were attempts to inactivate microscopic fungi on barley grains by essential oils, but the method reduced their germination [35]. In literature, there are data corroborating that such physical disinfection methods as grain cleaning, thermal treatment, exposure to high frequency electromagnetic oscillation, electron flow, photo luminescence or ozone are among the most perspective and least harmful to environment [2, 3, 9, 10, 14, 36, 42, 45,]. Meanwhile, fulfilment of most aforementioned technologies is very expensive and the efficacy of some has not yet been fully investigated. Taking into account that recently the problems of human health, nutrition and environment ecology have become increasingly urgent, it is necessary to search for new ecologic means to clean grains from microorganisms. One such means could be ozone application in selected concentrations.

Ozone (O_3) is a strong oxidant [27, 37]. It could be employed for oxidation of many microorganisms and chemical compounds [19]. Ozone, from the ecological point of view is more valuable than chemical disinfection means. Its important property is that it does not reduce the nutritive value of cereals [13, 31]. The main advantage of this disinfection means is that after reaction with microorganisms there are no harmful metabolites [16, 20]. Ozone molecules, before decompounding, stay in the air from 20-50 min, and in water – from 1-10 min [29]. For a long time, ozone has been used for water disinfection [20] and reduction of microbiological infection in the air of premises [40]. In addition, ozone acts simultaneously both as insecticide and fungicide [18, 21]. Researches on ozone effect on microscopic fungi damaging barley grains revealed positive effects without reducing their germination. Micromycetes mycelium is less resistant to ozone effect than conidia or spores [1]. It is stated that exposure of grains to ozone at a concentration of 5 ppb can stop the activity of *Aspergillus flavus*, known as a producer of aflatoxin [29]. It is noted that ozone is also effectively employed for detoxification of mycotoxin infected products [28, 30]. It has been established that ozone induces the grain drying process; therefore its proper application could reduce the grain drying duration by 25% [14, 41, 43]. It is stated that low ozone concentration (20÷30 $\mu g \cdot m^{-3}$) positively affects both plants and animals. Meanwhile, a concentration of ozone higher than 180 $\mu g \cdot m^{-3}$ is dangerous to human health [38]. Therefore, the problem of ozone application for grain disinfection should be solved and closely linked to the correct selection of ozone concentration, exposure duration, grain mound height and moisture content, as well as the concentration of outgoing ozone. One of the main factors is the intensity of ozone dispersion and absorption in the grain mound.

Work expedience: to investigate the efficacy of ozone exposure intensity in grain of different moisture content, and to establish parameters securing the safe application of ozone as a physical preventive means for reduction of mycological infection on grain surface.

METHODS

Investigations of the efficiency of physical means (ozone) for reduction of mycological pollution of grain surface were carried out in 2004-2005 in the Laboratory of the Heat and Biotechnological Engineering Department of the LŽŪU (Lithuanian University of Agriculture).

For investigations, a test rig was used (Fig. 1) consisting of: centripetal air blower 1, chamber with constant static pressure 2, connected with cylinders 5. Each cylinder of 0.18 m in diameter and 1.2 m in height contained 22.0 kg of wheat grain. Parameters of air supplied for their drying and ventilation intensity were the same for each cylinder. The same (0.12 ± 0.02 $m \cdot s^{-1}$)

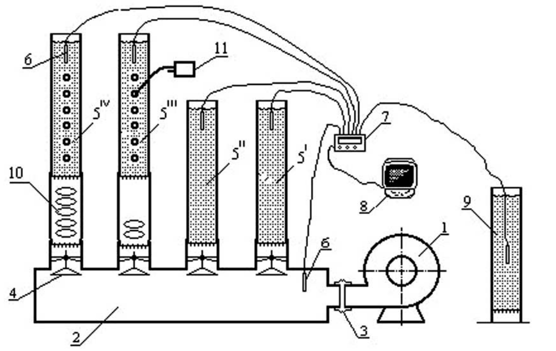

Figure 1. Schematic diagram of drying test rig: 1 – air blower; 2 – chamber with constant static pressure; 3 – flexible joint; 4 – restrictor; 5^{I}, 5^{II}, 5^{III}, 5^{IV} – ventilated cylinders with grain; 6 – temperature sensors and moisture content; 7 – secondary ALMEMO measurement unit; 8 – computer;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

velocity of air seepage through the layer of grain was chosen for each cylinder by regulation of the position for constrictors 4.

Mass value of grain dried was registered every 4 hours by weighing cylinders 5. The air temperature and relative humidity were measured using the ALMEMO sensors FH A646–21 (temperature data error ±0.1ºC, relative humidity error – ±2%). Measurement results were sent every 10 min to the data accumulating device ALMEMO 3290.

Investigation of ozone influence on the grain drying process. To determine exposure to ozone influence upon grain drying intensity, two ozonators were mounted at the bottom of third cylinder, while at the bottom the fourth cylinder – five ozonators 10. At established seepage velocity (0.12 $m \cdot s^{-1}$), each ozonator produced an ozone concentration in the air supplied for drying amounting to 140±5 ppb. Test rig cylinders were filled with "Tauras" wheat grain from the winter crop with an initial moisture content of 23.2±0.3%. Wheat was harvested and drying was commenced on the same day in the active ventilation rig. Grain was ventilated for 8 days, 8 hours daily (from 11.00-19.00). During the rest of the day, the test rig was inoperative and grain was not supplied to the cylinders because of increased humidity of the surrounding air. Grain was ventilated until its average moisture content in the cylinder diminished to 14%.

The natural ventilation cylinder 9, placed near the test rig, was filled with a control sample amount 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, the height of the grain mound inside was less than in the actively-ventilated cylinders, reaching 0.8 m. The temperature was measured in the centre of the grain mound in this cylinder.

Investigation of ozone permeability in the mound of grain exposed to ozone. Grains of "Tauras" wheat, having a different moisture content (*w*) was poured into the cylinders of the experimental rig: into the first 5^{I} and the fourth 5^{IV} – w=22.0±0.4%, while into the second 5^{II} and the third 5^{III} – w=15.2±0.1%. Five ozonators 10 at the bottom of the third 5^{III} and the fourth 5^{IV} cylinders each were mounted. Grain was ventilated daily for 5 hours, for 5 days (daily from 12.00-17.00). During the remaining hours of the day the rig was switched off. Ozone concentration in the grain mound (in cylinders 3 and 4) were recorded every 15 min using measurement device 11 (AHLBORN Ozon-Sonde FY A600-03). Ozone penetration rate and absorptivity was established from its variation. Initial micromycetes propagules number for 22.0% moisture grain was $M_0=1.1\times10^4\pm1.5\times10^3$ $cfu \cdot g^{-1}$, while for 15.2% – $M_0=7.1\times10^3\pm1.3\times10^3$ $cfu \cdot g^{-1}$.

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

Grain contamination with micromycetes propagules was established by dilution method. Grains, 10 g of each sample, were floured and diluted in 90 ml of sterile water, and then stirred for 10 min. The obtained flour suspension of 0.1 ml was spread onto Petri dishes on agar media. Flour suspension of grain, contaminated to a great extent with micromycetes propagules was repeatedly diluted 10 times. After 3, 5 and 7 days, the grown micromycetes colonies were counted and purified by inoculation onto 3 standard agar media: malt, Czapek and maize extract.

Contamination of grain and seed surface by micromycetes was evaluated by modified physiological solution of outwash [44], or applying Smirnova's and Kostrova's methodology [39].

Data obtained by investigations were evaluated using the methods of dispersion and correlation – regressive analysis.

RESULTS AND DISCUSSION

During research, the years grains of winter wheat and other cereals grown under Lithuanian ecologic conditions during harvesting were 100% contaminated with micromycetes. At the very beginning of storage, on the surface of grains and inside there were detected a number of micromycetes species – potential producers of toxic metabolites: *Alternaria alternata*, *Fusarium avenaceum*, *F. graminearum*, *F. poae*, *F. solani*, *F. tricinctum*, *F. sporotrichioides*, *Penicillium aurantiogriseum*, *P. aurantiocandidum*, *P. expansum*, *P. funiculosum*. In a later storage period the micromycetes of *Penicillium verrucosum*, *P. variabile*, *P. expansum*, *Aspergillus clavatus*, *A. niger*, *Bipolaris sorokiniana*, *Rhizopus oryzae* prevailed, *Fusarium poae* and *F. sporotrichioides* significantly reduced, *Alternaria alternata* was identified slightly less, and *Cladosporium cladosporioides* was more abundant on grain surface. The data reflects only summed-up results, but often in each grain batch or mound different micromycetes species were detected.

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

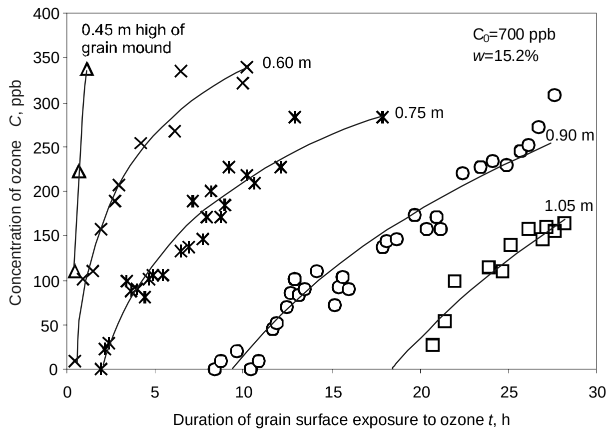

Figure 2. Variation of ozone concentration C at height of grain mound h as a function of exposure to ozone duration t, when the initial number of micromycetes propagules was M_0=7.1×10^3 cfu·g^{-1}.

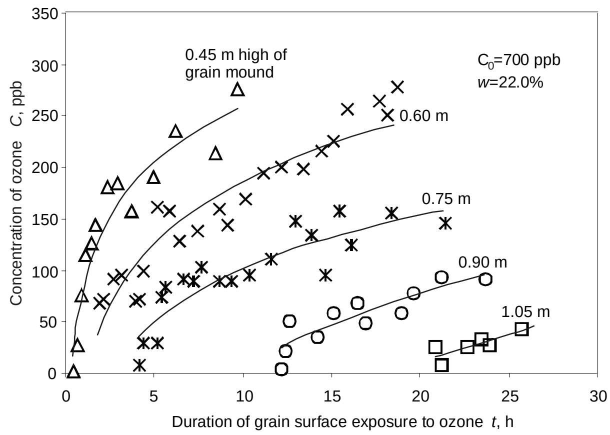

Figure 3. Variation of ozone concentration C at height of grain mound h as a function of exposure to ozone duration t, when the initial number of micromycetes propagules was M_0=1.1×10^4 cfu·g^{-1}.

move through pores from points of greater concentration to points with a smaller concentration, along the concentration gradient until the distribution balance is reached.

Penetration of ozone may then be expressed by the kinetic – diffusion equation:

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

where: C – ozone concentration; D – diffusivity; r – radius of the bottom of the grain mound; 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 the transversal direction while the second member evaluates diffusion in the longitudinal direction. The second member of the right side of the equation (1) evaluates ozone penetration because of its velocity, and the last member on the right side of this equation evaluates ozone absorption by the grain surface. If ozone moves into the grain layer together with the air supplied by the ventilator, that is, having a certain velocity and pressure, in the equation (1) the member representing diffusion of the ozone molecules may be neglected. Then the general kinetic equation for ozone penetration in the grain mound is:

$$\frac{\partial C}{\partial t} = -kC\ . \qquad (2)$$

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

$$\frac{\partial C}{\partial t} = -k\,C\,S\ . \qquad (3)$$

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

$$\mathrm{v} = \frac{\partial C}{\partial t} = \frac{\mathrm{v_f}}{\mathrm{V}}\mathrm{S}(\mathrm{C_0} - \mathrm{C}) - k\,C\,S\ , \qquad (4)$$

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

The first member on the right side of the equation (4) evaluates variation of concentration in time in case of absence of absorption (3), while the second member – absorption of ozone in reaction with the grain surface.

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

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

The magnitude of the ozone absorption factor (absorptivity) k can be determined in 2 ways: by substituting in the equation (4) experimentally-obtained ozone concentration values in separate moments of time, or by solving equation (4) by iterations.

In the case considered, the k factor numerical value was determined experimentally. Ozone concentration was registered periodically (every 15 min) (Fig. 2 and Fig. 3) and the penetration rate v was calculated (Fig. 4). Substitution of these data into the equation (5) allows determination of the absorption factor k (absorptivity), values of which are shown in Figure 5.

Experimental investigations confirmed the conclusions of the researchers [18, 24, 43], that ozone penetration is influenced mainly by the exposure to ozone duration (Fig. 2 and Fig. 3).

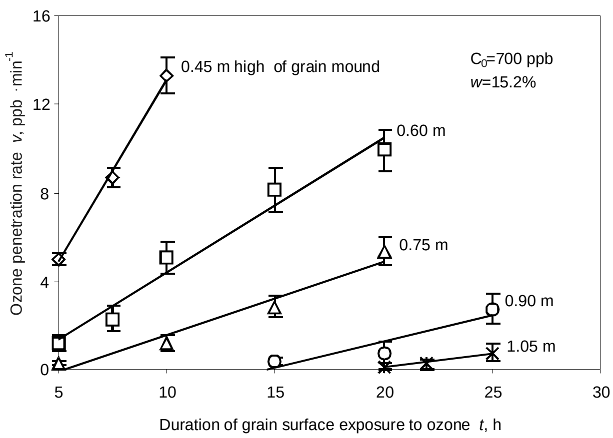

Figure 4. Dependence of ozone penetration rate v on grain surface exposure to ozone duration t, when initial number of micromycetes propagules number was M_0=7.1×10^3 cfu·g^{-1}.

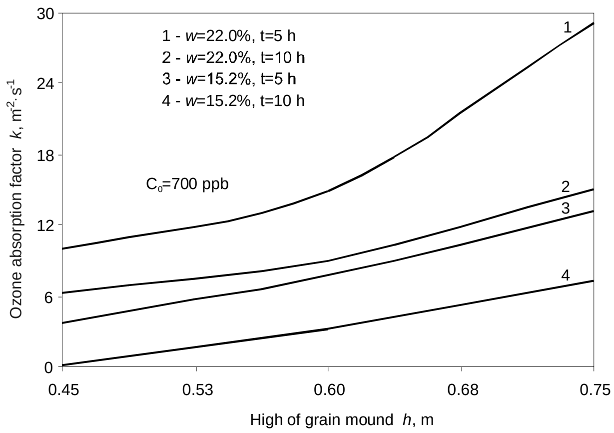

Figure 5. Influence of grain moisture *w*, height of mound (column) *h* and exposure to ozone duration *t* on absorption factor *k* of ozone.

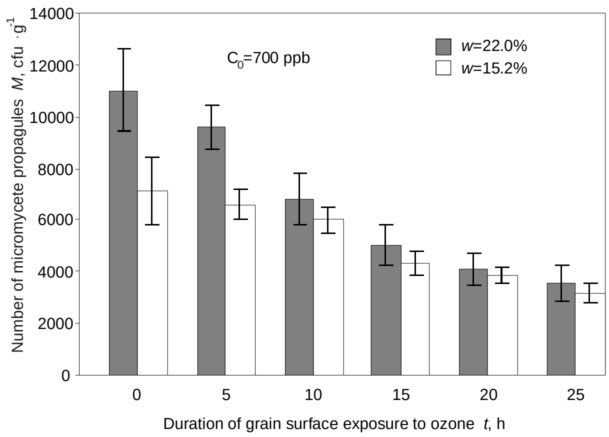

Figure 6. Influence of wheat grain moisture *w* and exposure to ozone duration *t* on mycological pollution of their surface.

In addition, ozone penetration depends also on the properties of the products ozonated [19]. With greater exposure to ozone time, ozone presence was registered each time at a higher mound layer. In addition, variation character of ozone concentration depended on the grain mound (column) height. At the beginning of the process, ozone was penetrating (up to 0.60 m) bottom grain layers very rapidly, later concentration increased at a lower rate (Fig. 2). At mound heights over 0.75 m, ozone concentration growth was less intense, being more gradual. Ozone concentration variation character in the mound with greater grain moisture (*w*=22.0%) (Fig. 3) resembled variation character for grain with *w*=15.2% moisture (Fig. 2). However, ozone at the same mound height was registered in the case of greater moisture later, if compared with drier grain. 15.2% moisture ozone at 0.60 m height was registered already in grain after 0.5 h, and 0.75 m after 2.5 h (Fig. 2), at 22.0% – after 2 h and 4.5 h (Fig. 3) correspondingly. An even greater time difference (about 4 h) was obtained by comparing the beginning of ozone registering in the upper layers (0.90 m and 1.05 m) of the mound.

During exposure of the grain surface to ozone, its absorption was observed [45]. Mendez *et al.* noticed that the ozone penetration rate *v* for grain mound is not constant [31]. Our investigations indicate that it is directly proportional to duration *t* of the exposure to ozone, and depends on the grain mound height coordinate *h*. After analysis of ozone penetration (concentration) measurement data, which was measured every 15 minutes during the whole period, ozone penetration rate *v* was established and its variation diagram drawn (Fig. 4). It was observed, that rate *v* each following day (every 5 h) at the same grain mound height, was greater. After 2 days (10 h of ozonating) at 0.60 m height, 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 increased slower (at 0.75 m height after 10 h it reached the value of 1.19 ppb·min^{-1}). According to data presented in Figure 4, it is safe to suggest that in ozonated grain, the ozone penetration and absorption rates are greater, while absorption is less.

It was found in previous works of various researchers, that ozone absorption in the grain layer depends on ozone concentration in air supplied to the layer, and exposure to ozone duration [24, 44, 45], velocity of air supplied [18, 31] and also on temperature [1]. In addition, Allen *et al.* states that the intensity of ozone absorption leads to conclusions about pollution of the grain surface [1]. Authors underline that the greater the mycological pollution, the slower ozone penetrates into the grain medium.

By substitution of obtained values of ozone penetration rates v into equation (5), ozone absorption factor *k* was calculated (Fig. 5). Experimental investigations carried out in 2005 showed that ozone absorption in the grain layer is influenced not only by the parameters mentioned above, but also by grain moisture *w* and mound height *h* (absorption is greater in upper layers of the mound). As the mound of ventilated grain begins to dry from the bottom layers [47], and with grain drying and moisture content diminishing, numbers of microorganisms also diminish, ozone absorption in the upper layers starts increasing.

It was established that ozone, in reaction with the more moist grain surface, the mycological pollution of which is usually higher than that of a dry one splits more rapidly. As ozone is a strong oxidizer, at the same time it destroys microorganisms of the grain surface, including mould fungi. Thus, in the case of more moist grain, ozone penetrates its layer slower, with a longer reaction with the grain surface and microflora on this surface, the destruction of which is more effective because of longer exposure.

Grain drying by active ventilation is a cheap and rather universal drying procedure which enables reduction of the harmful effects of microorganisms [47]. Investigation revealed that the number of micromycetes propagules in unventilated grain grew insignificantly during the first 2 days (by 20%); however, after 5 days it nearly doubled compared with the initial value (1.9×10^4 cfu·g^{-1} vs. 1.1×10^4 cfu·g^{-1}).

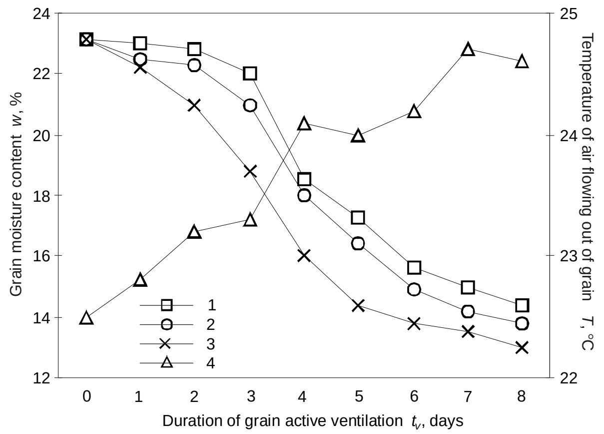

Figure 7. Influence of wheat grain "Tauras" ventilation duration t_v on variation of grain moisture *w*: 1 – moisture of grain ventilated without ozone; 2 – moisture of grain ventilated, when ozone concentration is 280 ppb; 3 – moisture of grain ventilated, when ozone concentration is 700 ppb; 4 – average temperature of air flowing out of grain.

In addition, in grains untreated with ozone, the species diversity was greater: in non-ventilated grains there were identified micromycetes of 26 species, and in ventilated grains – only 11 species.

Application of ozone in grain drying by active ventilation can stop not only development of microscopic fungi, but reduces their abundance and change their composition. Investigations revealed that mycological grain surface pollution was reduced to a greater extent while ozonating the grain mound with greater grain moisture content (Fig. 6) with greater ozone absorption. Mycological pollution of more moist (22.0%) grain during exposure to ozone for 25 h was reduced about 3 times, while mycological pollution of less moist (15.2%) grain – 2.2 times. During active ventilation, when the ozone amount 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}. This confirms assertions that ozone in media with a greater surface moisture content is a more active oxidizer [1, 20]. With 280 ppb ozone concentration in the drying agent, the change of grain mycological pollution, compared with ventilation without ozone in air, was not statistically significant.

On the basis of investigation results obtained, it is safe to suggest that the 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 of ensuring the feasibility of the application of ozone.

Investigation of ozone influence upon grain drying process. It was determined by investigations that ozone used together with a drying agent also supports the grain drying process, particularly during the first 3 days (Fig. 7).

In the case of a drying agent without ozone, grain moisture diminished after 3 days from 23.2-22.1% (Fig. 7, curve 1), while with 700 ppb concentration in air – from 23.2-19.0% (Fig. 7, curve 3). Later, grain drying intensity differed only slightly; however, at ozone concentration 700 ppb grain moisture of 14% was reached 2 days earlier than in the case of air without ozone. Drying agent with ozone concentration of 280 ppb had little influence on the drying process. More intense reduction of grain moisture with surface exposed to ozone is possible because of the interaction of ozone with the water or moisture content of material exposed, causes changes in the physical, chemical and thermal properties of water [13]. In addition, the drying process is considerably influenced by the temperature of the drying agent reaching 24±1.2ºC. During the drying process, the temperature of the air flowing out of grain became almost equal to the surrounding air temperature (Fig. 7).

Changes in micromycetes species diversity were observed during ventilation by ozone – air mixture. Micromycetes of *Fusarium*, *Geotrichum*, *Myrothecium* and other species stopped active functioning. Micromycetes of some species of *Penicillium* and *Aspergillus* genera like *Penicillium verrucosum*, *P. cyclopium*, *Aspergillus niger* and *A. clavatus* persisted in existing. Fungi of *Alternaria alternata* and *Mucor* genera were reduced significantly. Fungi of *Cladosporium cladosporioides* species increased insignificantly. The abundance of micromycetes propagules producing mycotoxins in air and on an ozone dried grain surface was reduced, but propagules were not totally exterminated. Micromycetes of some species survived. They can produce and ooze-out into the environment: mycotoxins: ochratoxins, patulin, cytochalasin, malphormin and others dangerous to human health.

CONCLUSIONS

1. Investigations revealed that with an air-ozone mixture blown through the grain mound, ozone splits in contact with the grain surface while disinfecting 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 upper layers of the grain mound ventilated. A direct link exists between grain moisture and ozone absorption, and more moist grain has greater absorption.

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

3. An ozone-air mixture used for active ventilation-drying of grain (with an ozone concentration of 700 ppb) enables the reduction of drying duration by about 20%, and reduction of mycological pollution depending on moisture content *w*, from 2.2 times (for grain *w*=15.2%,) to 3 times (for grain *w*=22.0%).

4. An ozone and air mixture inhibits or retards significantly development on grain surface of fungi belonging to *Fusarium*, *Geotrichum*, *Myrothecium* and *Mucor* genera, while fungi of *Alternaria* and *Verticillium* genera are partially inhibited. Micromycetes of various

species of *Penicillium* and *Aspergillus* genera more often survive in ventilated grains.

5. Ozone is a reliable means for reducing the mycological contamination in raw food material obtained from cereals, and to avoid danger to human health caused by micromycetes that synthesize toxic secondary metabolites and ooze them out into environment. It is necessary to improve the efficacy of ozone technologies, and to expand the application range in order to preserve healthy food from mycological contamination for a longer time, and to improve sanitary conditions.

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