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Preventive measures reducing superficial mycobiotic contamination of grain

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Abstract

Search for the preventive measures reducing the accumulation of mycotoxin producers in food raw material was carried out. Active ventilation was used; the impact of the electro-chemically activated air (ozone) and electro-chemically activated water (anolyte) on the micromycetes prevailing in grain raw material for food (GRMF) was determined. The GRMF was dried by active ventilation using the ozone-air mixture. Ozone (concentration 1250 ppb) disinfects the surface of the raw material and creates conditions unfavourable for the increase of mycobiotic contamination in drying upper layers of the grain mound. Within 8 days the contamination of GRMF in a mound decreased by 50%, while in its lower layers – more than 3 times. Ventilation of the mound with the above-mentioned concentration of the ozone-air mixture has ceased the active functioning of *Fusarium avenaceum*, *F. graminearum, F. poae, F. solani, F. tricinctum F. sporotrichioides* micromycetes and has considerably retarded the development of *Alternaria alternata* and other fungi. Anolyte (0.05% of chlorine concentration) reduced the mycobiotic contamination of GRMF by almost 2.5 times. The optimal treatment duration is from 0.5 to 1 hour. The optimal technical parameters, allowing the use of these measures for the preparation of grain food safety technologies, were elaborated; they are designed for more efficient protection of human health against micromycetes and their toxic metabolites, which are abundantly produced and released into the environment.

Key words

contaminated wheat, micromycetes, mycotoxins, ventilation, ozone, anolyte

INTRODUCTION

The data presented in literature references reveal that annually about 25% of the grain yield is being contaminated with micromycetes that produce and release into the environment mycotoxins – toxic substances of various chemical composition. These metabolites constitute toxigenically and chemically heterogeneous assemblages that are grouped together only because the members can cause disease and death in human beings and other vertebrates [1]. There are currently investigated more than 350 species of micromycetes, about 300 of their able to synthesize mycotoxines, which degrades the quality of food and pose a risk to human health [2]. In order to prevent diseases and to achieve beneficial balance in nature, the micromycete activities must be strictly controlled at all stages of grain raw material processing – from the plant sowing to the end-product delivered to the consumer. Bata and Lásztity [3] state that the best results in avoiding the risk of mycobiotic contamination of raw material is the application of a wide range of preventive measures.

Currently, various regulatory measures, i.e. agrotechnical, technical, agrochemical, chemical, biological and microbiological, are used to reduce the mycobiotic contamination of grain raw material for food (GRMF). One of the simplest and most commonly used technical measures is drying by active ventilation. Selection of other appropriate measures is complicated because no human health-friendly, biologically and economically effective measures for detoxification of grain raw material have been elaborated yet. Therefore, in order to reduce mycobiotic contamination of food raw material and improve people's safety, the integrated control methods must be applied [4, 5].

According to the research results published by scientists from different countries, the following classification scheme of the preventive measures used to reduce mycobiotic contamination could be compiled (Fig. 1).

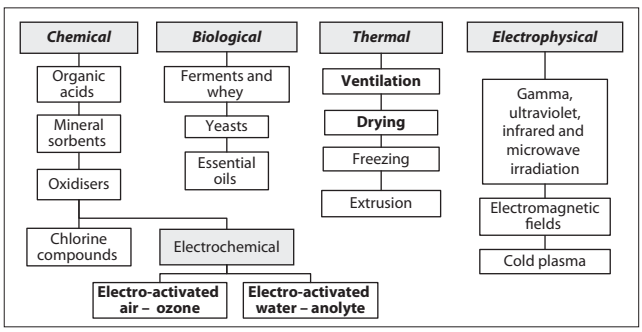

Figure 1. Search for preventive measures reducing the mycobiotic contamination of grain raw material for food

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Electro-activated air (ozone O_3) is attractive because its degradation product is oxygen, thus leaving no undesirable residue [6]. Ozone is generally recognized as safe by the Food and Drug Administration of the USA [7, 8]. It is a very strong oxidiser; ozone oxidation potential (2.07 eV) is much higher than of hydrogen peroxide H_2O_2 (1.78 eV), chlorine Cl_2 (1.36 eV) or oxygen O_2 (1.23 eV) [9]. Both ozonized air and ozonized water are used for microorganism inactivation [9], but under the impact of gaseous ozone the oxidative reactions are significantly faster than in liquid [10].

Ozone can destroy not only bacteria and viruses [11, 12] but also micromycetes [13]. It has been determined that microorganism species are characterized by different resistance to ozone [14]. Besides, micromycetes are much more resistant to ozone than other microorganisms [15]. It is stated that less than 0.003 $mg \cdot l^{-1}$ ozone concentration has no effect upon micromycetes, concentration exceeding 0.03 $mg \cdot l^{-1}$ suppresses their propagation and growth, the ozone concentration of about 1.5 $mg \cdot l^{-1}$ destroys their vegetative systems, and the concentration exceeded 20 $mg \cdot l^{-1}$ destroys micromycete propagules (conidia, spores and other colony forming units (CFU)) [16]. Some researchers state that higher ozone concentrations can destroy the mycotoxins: aflatoxin [17], patulin [18], deoxynivalenol [19] and moniliformin [20]. It is noted that during the reaction of ozone and some mycotoxins (aflatoxin B_1 and fuminozin B_1) new products may form, and they could possess mutagenic effects [17]. It is also noted that after ozonation of aflatoxin-free GRMF, no harmful products formed [17], therefore the ozonation should be performed prior to mycotoxin formation in a mound of raw materials.

Successful suppression of the development of ozonized micromycetes depends on the ozone concentration and ozonation time [21, 22]. It has been noted that, if after 36 hours of ozonation with very low concentrations – 50 ppb and 100 ppb – the abundance of *Rhizopus stolonifer* micromycetes decreased by 57% and 76%, and of *Botrytis cinerea* – by 68% and 78%, then the increased concentration of 1000 ppb reduced the amount of micromycetes by 99% [23]. There are statements that 300 ppb ozone concentration can inhibit mycelium growth and sporulation of *Monilinia fructicola, Botrytis cinerea, Mucor piriformis* and *Penicillium expansum* on the surface of the fruit preserved for 4 weeks at 5°C and 90% relative ambient humidity conditions [24]. It is noted that sporicidal properties of ozone depend on the relative humidity; as the humidity decreases, the time required to destroy microorganism propagules increases [25]. During 23 hours at 90% relative air humidity ozone almost completely destroyed conidia of *Penicillium chrysogenum* [26]. Not all micromycete species react equally to identical ozone concentrations. Evident decrease of the conidia formation of *Penicillium italicum* held for 5 days at 20°C and previously treated for 4 days with 300 ± 50 ppb ozone concentration at a temperature of 5°C was recorded. Such treatment, however, had no significant effect on the conidia formation in *Penicillium digitatum* [27]. *Aspergillus flavus* and *A. niger* micromycetes are characterized by exceptional resistance to ozone [28]. It has been also noted that micromycete conidia and spores are more resistant to ozone than mycelium [29].

Reduction of GRMF contamination with mycopropagules remains a topical issue in developing effective measures to protect human health against adverse environmental factors. The literature references concerning the use of ozone for GRMF purification are rather numerous, but effective and secure technological solutions are still inadequate; the issues of ozone and grain raw material interaction are poorly studied; the influence of environmental factors on this interaction is not thoroughly investigated. All this leads to the necessity of the studies devoted to various concentrations of ozone used for grain raw material safety, search for more efficient variants of ozone use, optimize the existing and develop new food safety technologies considering the ozonation time, effectiveness, benefit coefficients and a range of technical opportunities, which arise from the specific conditions of food production, evaluation of raw material, meteorological factors and economic contexts.

Studies on **electro-chemically activated water (anolyte)** demonstrated its bactericidal, antiviral and partially fungicidal properties [30]. Recently extensive studies on the use of electro-chemically treated water for the reduction of mycobiotic contamination during the harvest processing have been widely performed in Japan, the United States, China and Russia. It was found that the anolyte is characterized by mycostatic and, especially, bacteriostatic effect on micro-organisms, continuing for a 24-hour period [31]. This water was used in post-harvest mycological safety of fruit and vegetables, replacing chemical fungicides [32]. According to the disinfecting properties, anolyte could be placed between chlorine and ozone [33]. It has been determined that although the anolyte possesses fungicidal properties, but has different effect on various micromycetes. Among the most important factors determining the fungicidal activity of the anolyte are hydrogen peroxide H_2O_2 content [34], oxidation-reduction potential and pH [35]. Within 20 minutes activities of *Aspergillus*, *Cladosporium* and *Penicillium* spp. micromycetes were suspended, meanwhile the growth of *Tilletia indica* (syn. *Neovossia indica*) has intensified [36]. It should be noted that fungi of the latter genus are active agents of wheat smut. The data on the study of the resistance of 22 fungal species to anolyte revealed that propagules of such genera as *Botrytis, Monilinia* were destroyed during 30 s, and the development of *Curvularia, Helminthosporium* was significantly suppressed [37]. During the 30-minute period not only 95% of micromycete propagules present on the GRMF surface were destroyed, but the content of mycobiots within the raw material was reduced by 21% [33]. Therefore, in order to destroy micromycetes within the GRMF, significantly longer duration of anolyte treatment is required. It is noted that products baked using the anolyte-treated supplies are characterized by better organoleptic properties [38].

Studies have shown that after entering the animals' organism the anolyte does not harm the main organs and has no mutagenic effect [39]. Therefore, the application of electro-chemically activated water could be a promising, cheap, environmentally friendly and easily technologically adaptable food safety measure, but further research and development of effective application technologies is needed for its wider application [30, 32].

Overview of the literature references and abundance of the used measures indicate that the issue is very important, closely related to food safety and human health. Analysis of the published research data suggests that currently electroactivation-based technological solutions, i.e. application of electro-activated air (ozone) and the electro-chemically activated water (anolyte), can be considered among the most affordable measures for reduction of mycobiotic contamination of grain raw material.

The aim of the work was to evaluate the efficiency of active ventilation and electro-chemical tools: ozone and anolyte, in reducing the contamination of grain raw materials for food with mycopropagules. Also to choose the optimal technical parameters and conditions for application of these measures and to use them in preparation of new technologies aimed to ensure the safety of grain materials for food and protection of human health against micromycetes and their abundantly produced and released toxic metabolites.

MATERIALS AND METHODS

Research on preventive measures for reduction of mycobiotic contamination of grain raw materials for food were conducted at the Laboratory of agricultural products storing and processing technologies of the Department of Heat and Biotechnological Engineering at the Lithuanian University of Agriculture.

Investigations on GRMF drying by active ventilation with ambient air and ozone-air mixture. The setup used for this investigation consisted of centrifugal ventilator „KVKE 250 L TW" (Systemair AB, Sweden), chamber of constant static pressure and five ventilated cylinders [21]. Simultaneously GRMF of wheat variety "Tauras" was dried with ambient air in two 1.2 m high and 0.18 m in diameter cylinders using the same ventilation rate velocity (v_f = 0.06 ± 0.01 m·s^{-1}, v_f = 0.12 ± 0.02 m·s^{-1} or v_f = 0.24 ± 0.05 m·s^{-1}). Before the grain drying, ventilation velocity was adjusted with valves present at the bottom of each cylinder. Each cylinder contained 22.0±0.5 kg of wheat grain. During the study, the mass of drying GRMF was recorded by weighing the cylinders with mechanical scales „RP-200Š13" (Russia) every 4 hours. Air temperature and relative humidity were measured by ALMEMO sensors FH A646-21 (temperature reading error ±0.1°C, relative humidity error – ±2%). Measuring results were stored in the secondary device ALMEMO 3290 (Ahlborn Mess- und Regelungstechnik GmbH, Germany) every 10 min.

For GRMF drying with ozone-air mixture, other two drying cylinders with ozonators located under them were used: two ozonators were placed under one of them and five ozonators – under the other. In the incoming air the concentrations of the produced ozone were 250 ± 10 ppb, 500 ± 10 ppb or 1250 ± 10 ppb. The concentration of ozone produced by each ozonator was 250 ± 10 ppb. Ozone concentration of the grain raw material (initial moisture content 23.2 ± 0.3%) mound was recorded by ozone meter AHLBORN Ozon-Sonde FY A600-03 (Ahlborn Mess- und Regelungstechnik GmbH, Germany) and „GasAlertmicro" (BW Technologies, Calgary, Canada) alarm sensor (C_{max} = 1300 ppb, measuring error ± 10 ppb). GRMF was being ozonized continuously (ozone concentration 250 ppb, 500 ppb and 1250 ppb) or periodically for 2 h a day (ozone concentration 500 ppb).

The remaining cylinder, i.e. natural ventilation cylinder, was located in the same room next to the setup. It was mounted on a 10 cm high grid so that the ambient air could enter the mound of the grain raw material. The mound height was lower than in other ventilated cylinders and reached 0.8 m. The temperature was measured in the central part of the grain material the cylinder.

GRMF was ventilated for 8 days. Its initial mycobiotic contamination was $M_0 = 6.1 \times 10^3 \pm 0.9 \times 10^3$ CFU·g^{-1}, $M_0 = 8.3 \times 10^3 \pm 1.5 \times 10^3$ CFU·g^{-1} and $M_0 = 9.2 \times 10^3 \pm 2.8 \times 10^3$ CFU·g^{-1}.

Electro-chemically activated water (anolyte) for GRMF treatment. This was produced in a special electro-chemical activation device STEL (NPO Ekran, Russia) where tap water and table salt NaCl were supplied. The device is a container divided into anode and cathode chambers by a semi-permeable membrane (Fig. 2).

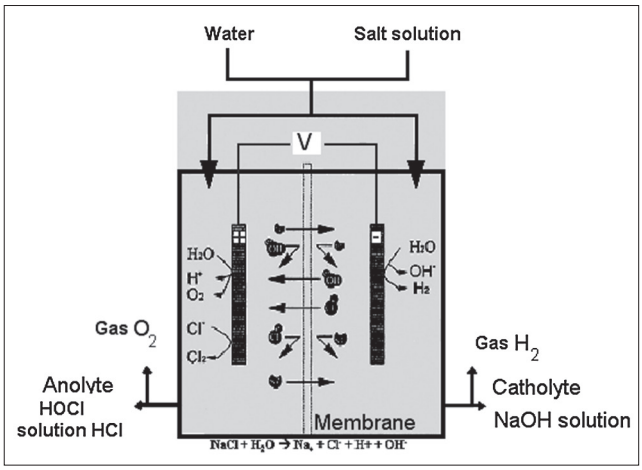

Figure 2. Device for electro-chemical activation of water [31]

Electro-chemical activation of the salt solution resulted in the production of anolyte in the anode chamber, and catholyte – at the cathode chamber. In order to determine the effect of anolyte on GRMF mycobiotic contamination, 100 g of the raw material was poured into the dishes with anolyte solution and mixed so that its surface is evenly covered with anolyte. The raw material was kept in dishes for 0.25 to 12 hours. Investigations were carried out with anolyte solution of three different chlorine concentrations: 0.01%, 0.02% and 0.05%; this corresponded to 100 mg·l^{-1}, 200 mg·l^{-1} and 500 mg·l^{-1} of active chlorine. In order to justify the oxidation-reduction potential limits applied during the research, the data of the below-listed literature references was used. Anolyte with pH ranging from 2.6 to 2.8 and oxidation-reduction potential of +1100 mV is usually acidic. It also contains hypochlorite HOCl, which shows strong bactericidal properties [40, 41]. Meanwhile catholyte is alkaline, the pH ranging from 8.0 to 10.4 and more, the oxidation-reduction potential being from -477 mV to -878 mV [38], therefore it is used in food industry as an antioxidant [42]. Besides, mycobiotic effect of alkaline electro-activated water (catholyte) is weak [30, 31]. This is explained by the fact that the constant electric current flowing through water decomposes and oxidizes it as well as reduces the impurities. Due to water reduction: $2H_2O + 2\bar{e} \rightarrow H_2 + 2OH^-$ hydrogen H_2 is released at the cathode (Fig. 2). Meanwhile oxygen O_2 is released at the anode due to the oxidation of water: $2H_2O \rightarrow O_2 + 4H^+ + 4\bar{e}$ [30, 31]. In addition, ozone, hydrogen peroxide, chlorine oxide, chlorine, hypochlorite, free radicals and other active compounds possessing disinfectant properties form in the anolyte (Tab. 1).

Data presented in Table 1 can be used to assess the anolyte performance against the GRMF mycobiotic contamination.

Table 1. Reactive ions and free radicals formed in the electrochemically activated anolyte [43]

Molecules	Ions	Free radicals
O_3, O_2, H_2O_2, ClO_2, HClO, Cl_2, HCl, $HClO_3$	H^+, H_3O^+, OH^-, ClO^-, Cl^-	OH, HO_2, O_2, O, ClO, Cl

Microbiological testing of raw material. Mycobiotic contamination of GRMF was determined by the direct spread plate and prints (appliqué) method [44]. The analyzed GRMF samples were placed in sterile Petri dishes on agar malt extract medium supplemented with chloramphenicol (50 ml·l^{-1}) and slightly pressed to the surface of the medium with a sterile scalpel. Petri dishes were kept in a thermostat at 26 ± 2°C with a lid to the top for 24 hours, later flipped upside down and kept changing the darkness and light regime every 12 hours. Sometimes the modified method was used: GRMF samples were placed in empty sterile Petri dishes and poured over with 48°C medium of the above-mentioned composition.

The flush-dilution method was used as follows: 1 g of GRMF was weighed in sterile conditions and poured into 9 ml of sterile water, shaken for 15 minutes and dilution series was prepared from the obtained suspension; 1 ml of the initial suspension was poured into 9 ml of sterile water and further dilutions of raw material were prepared. From each dilution series 1 ml of suspension was poured into sterile 9 cm diameter Petri dishes, with light hand movement spread at the bottom of the dish and poured over with 15 ml agar malt extract medium with chloramphenicol of 48°C. Microorganisms were cultivated in a thermostat at the above-mentioned conditions.

In order to purify the obtained micromycete cultures they were sown on agar malt extract, Chapek and corn extract media and further grown at 26 ± 2°C for 5-7 days. Micromycete species were identified according to numerous manuals [2, 45, 46, 47, 48, 49, 50, 51]. The isolated micromycetes were systematically grouped following Ainsworth & Bisby's Dictionary of the Fungi 8th Edition [52]. Detection frequency (%) of each identified species was calculated [53].

Least significant difference threshold (R). All experiments in this study were repeated in triplicate or more. In order to determine significance differences between means of tested variant data, the threshold value of significance difference $R_{0.05}$ was calculated [54]. Differences among means were compared by the one way Analysis of Variance (ANOVA) and using *t*-test with 95% confidence. Columns in figures followed by the same letter are not significantly different at 5% level according to *t* post hoc test. The experimental results were processed using the MS Office Excel program.

RESULTS AND DISCUSSION

Mycobiotic contamination of GRMF dried by active ventilation with ambient air. Active ventilation is one of the most technically affordable measures for raw material drying. While drying the GRMF with active ventilation it is particularly important not only to reduce the water content of raw material but also to prevent the increase of its mycobiotic contamination. Therefore, the influence of the ventilation intensity at the bottom and the top layers of the grain mound, i.e. the critical places of the dried material mound, was assessed and expressed as CFU g^{-1}.

When GRMF was kept in the cylinder with natural ventilation (non-ventilated GRMF), during 8 days the moisture content of wheat raw material almost unchanged (Fig. 3).

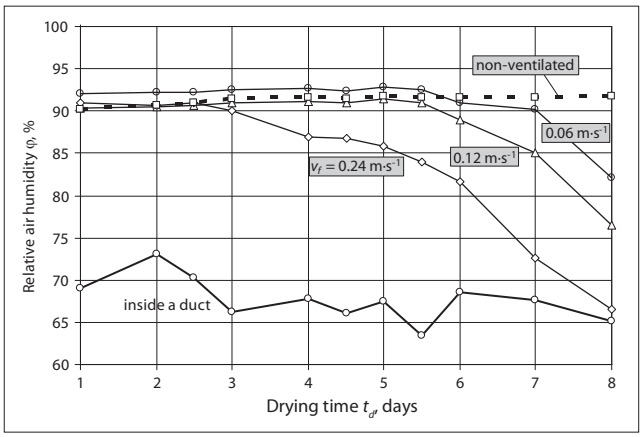

Figure 3. Impact of ventilation intensity on the relative humidity φ changes in the top layer of the active ventilation cylinder with the wheat grain: the initial abundance of micromycete CFU in raw material of 23.2 ± 0.3% moisture content, $M_0 = 8.3 \times 10^3 \pm 1.5 \times 10^3$ CFU·g^{-1}

The air flowing through the GRMF mound absorbed moisture, so the artificially ventilated material was drying more intensively. The drying depended on the air filtration velocity (m·s^{-1}), which is associated with the supplied air flow (m^3·(t·h)$^{-1}$). The raw material was continuously ventilated all through the day, even in spite of unfavourable meteorological conditions. The investigations revealed not just reduced GRMF weight or moisture content, but sometimes also their increase. Thus, the ventilated material may not only release but also absorb moisture from the flowing air, if high moisture content is present in the environment.

As the air filtration velocity increases, so does the GRMF drying rate. GRMF was most intensively drying in the cylinder with the highest air filtration velocity (v_f = 0.24 m·s^{-1}). At GRMF ventilation with 0.24 m·s^{-1} air flow velocity and 793 m^3·(t·h)$^{-1}$, supplied air flow, the relative humidity (φ = 75%) in the top layers of the mound was reached after nearly 7 days. At this relative humidity, the moisture content of the raw material was 14-15%. This means that the reduction of moisture content in raw material from 23.2 ± 0.3% to 14-15% took almost 7 days. When velocity of the air flow supplied into the raw material mound was reduced to 0.12 m·s^{-1}, the raw material reached the same moisture content within 8 days, and when v_f was only 0.06 m·s^{-1}, 8 days were not enough to reduce the humidity to 14–15%. The ventilated GRMF mound begins to dry starting from the bottom layer, so the moisture content there was decreasing more intensively. It has been determined that the moisture content is one of the key factors predetermining the development of micromycetes [55]. The faster is the evaporation of moisture from the GRMF, the faster moisture threshold at which micromycetes can not develop is reached. This statement is supported by the test results on mycobiotic contamination of the top and bottom layers of the GRMF mound (Fig. 4).

Higher air filtration velocity used for GRMF ventilation (0.12 m·s^{-1} and 0.24 m·s^{-1}) inhibited the micromycete

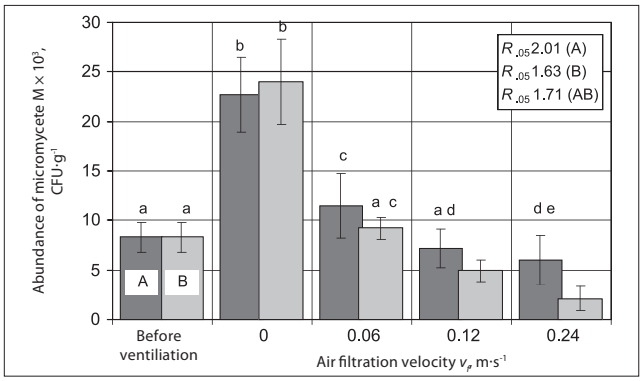

Figure 4. Abundance of micromycete CFU in the top (A) and bottom (B) layers of the active ventilation cylinder after 8 days of wheat raw material drying: the initial abundance of micromycete CFU at 23.2 ± 0.3% moisture content of raw materials, $M_0 = 8.3 \times 10^3 \pm 1.5 \times 10^3$ CFU·g^{-1}.
a, b, c, d, e no statistically significant difference at the 95% confidence probability level was recorded between the mean values of the columns marked with the same letter

development; at the air filtration velocity of 0.06 m·s^{-1} in the bottom layer of the mound mycobiotic contamination remained unchanged while at the top layer – slightly increased. Statistically significant differences between contamination of the bottom and top layers was recorded when 400 m^3·(t·h)$^{-1}$ (0.12 m·s^{-1}) comparative air flow was supplied. In the top layer of the raw material, the amount of micromycete propagules was about 50% higher than in the bottom GRMF layers (Fig. 4). Even larger difference was observed as the velocity of the supplied air was increased up to 0.24 m·s^{-1}. Then, after 8 days of ventilation, mycobiotic contamination of the top layers of the mound was $6.0 \times 10^3 \pm 2.5 \times 10^3$ CFU·g^{-1}, and of the bottom layers – $2.1 \times 10^3 \pm 1.2 \times 10^3$ CFU·g^{-1}. The data of this study as well as researches of other authors [56] show that further increase of ventilation intensity is ineffective and has no significant impact on the reduction of the number of micromycete propagules in GRMF.

During the study period, the number of micromycete propagules in non-ventilated GRMF increased three-fold (Fig. 4), because very favourable conditions for their development had formed. No statistically significant difference between mycobiotic contamination at the top and bottom of the GRMF mound has been identified.

In the top layers of the GRMF mound dried applying active ventilation, conditions favourable for micromycete development last for the longest period of time. Mycobiotic contamination of the ventilated GRMF depends on the volume and filtration velocity of the supplied air; its optimal value is 0.24 m·s^{-1}.

GRMF drying by active ventilation using ozone-air mixture. Studies were performed to determine the impact of ozonation and its parameters to ensure the safe use of ozone as a preventive measure in reducing the mycobiotic contamination of the GRMF. Winter wheat grain used in the ozonation investigations was heavily contaminated with micromycetes. A large variety of micromycetes was determined while harvesting. Certain micromycete species are ascribed to 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*, *P. verrucosum*, *P. variabile*, *Aspergillus clavatus*, *A. niger*, *Bipolaris sorokiniana* and *Rhizopus oryzae*.

Active ventilation of the GRMF with ozone-air mixture can not only halt the development of micromycetes, but also reduce their abundance. However, the effectiveness of ozone is closely associated with its concentration in the air supplied to the raw material mound. It is noted that ozonation of the GRMF with ozone-air mixture of low concentrations could increase its mycobiotic contamination (Fig. 5).

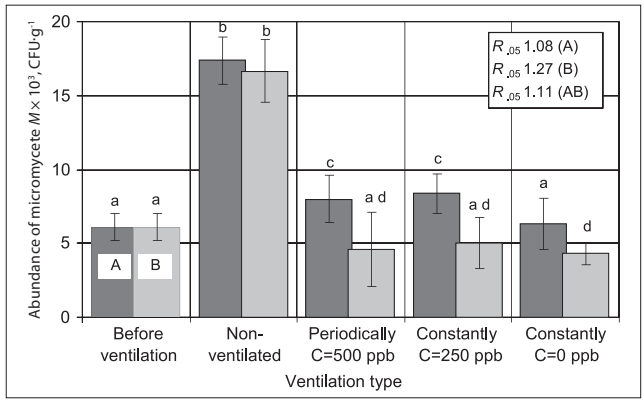

Figure 5. Abundance of micromycete CFU in the top (A) and bottom (B) layers of the active ventilation cylinder after 8 days of wheat raw material drying: air filtration velocity $v_f = 0.24 \pm 0.05$ m·s^{-1} ($Q = 793$ m^3·(t·h)$^{-1}$); $C_0 = 500$ ppb = 1070 µg·m^3; the initial abundance of micromycete CFU at 23.2 ± 0.3% moisture content of raw materials, $M_0 = 6.1 \times 10^3 \pm 0.9 \times 10^3$ CFU·g^{-1};
a, b, c, d no statistically significant difference at the 95% confidence probability level was recorded between the mean values of the columns marked with the same letter

In the GRMF ozonized for 8 days with 250 ppb (constantly) and 500 ppb (periodically 2 h per day) ozone concentrations the abundance of micromycete CFU was higher than in GRMF ventilated with ambient air. Periodic ozonation was chosen because some researchers [57] propose to apply ozonation not through the whole process of active ventilation, but only periodically – from 0.5 to 1 hour per day. However, both low concentration (250 ppb) and periodic ozonation (500 ppb) had an adverse effect on mycobiotic contamination of the GRMF. Other authors [58, 59] state that lower than optimal dose of disinfectants causes "stress mode" in microorganisms. Then their activity intensifies as a response to the disinfecting effect of the applied measure.

Studies have shown that ozone used together with the drying agent promotes only the beginning of the GRMF drying process. During the first 4 days, moisture content in the bottom layers of the GRMF mound ventilated with ambient air decreased from 23.2 ± 0.3% to 17.1 ± 0.2% (Fig. 6, a), ventilated with 500 ppb ozone concentration – up to 16.5 ± 0.1%, and with 1250 ppb – up to 15.4 ± 0.1% (Fig. 6, b, c). In the top layers of GRMF mound the moisture content decreased not so significantly: during 4 days moisture content of GRMF ventilated with ozone-free air almost unchanged, when 500 ppb ozone concentration was used the moisture content decreased only by 3 percentage points (to 20.7 ± 0.3%) and in case of 1250 ppb ozone concentration – up to 18.6 ± 0.3%.

Later the GRMF drying in all ventilation treatments was more intense, but 14% moisture content of the raw material was achieved almost simultaneously. According to the obtained data, drying agent containing ozone concentration up to 1250 ppb had no significant influence on the duration of the GRMF drying.

Some researchers state that ozone interacts with moisture of the ozonized substances and changes physical, chemical and thermal properties of water [57, 60].

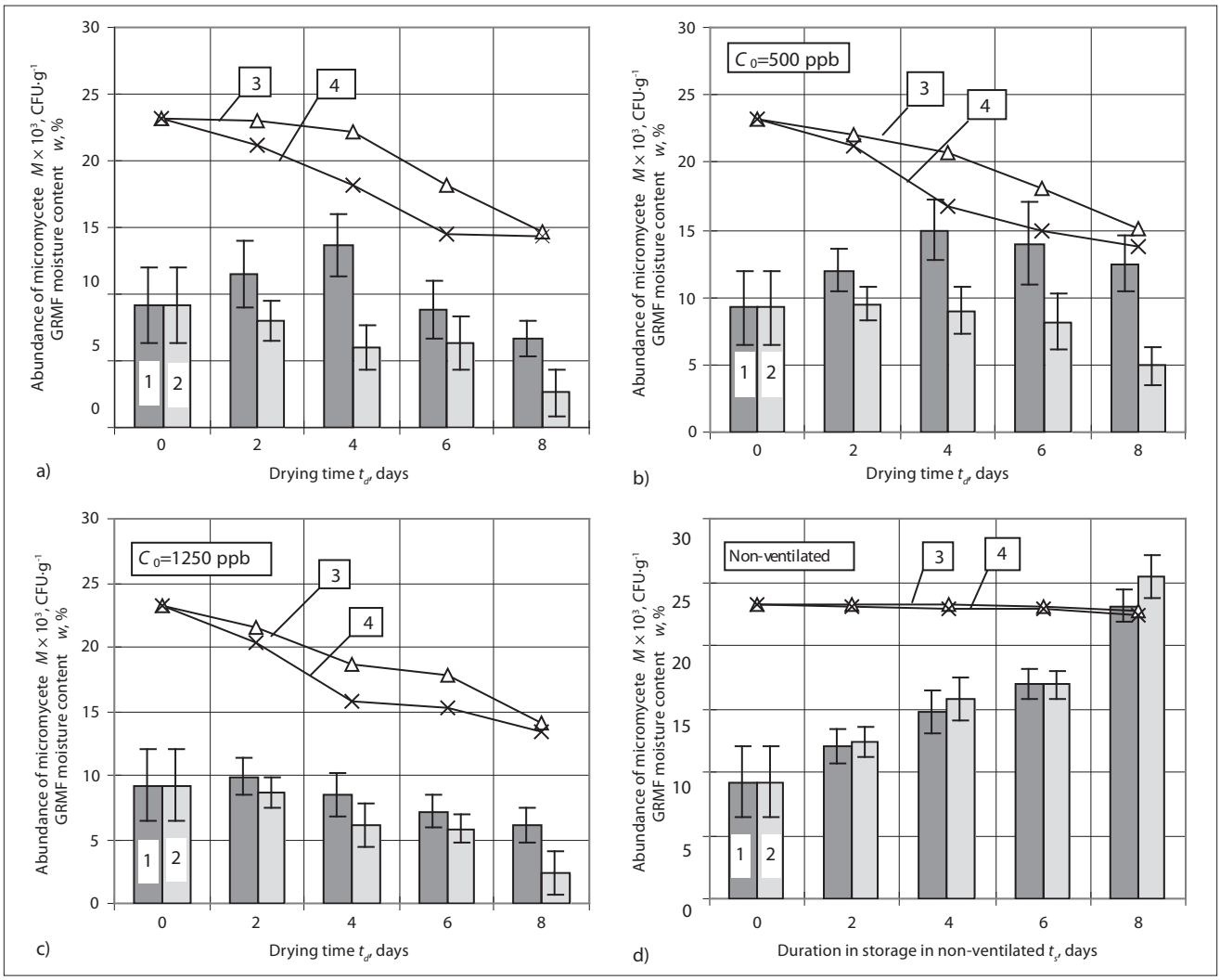

Figure 6. Changes of micromycete CFU in the active ventilation cylinder during the GRMF drying: 1 – CFU abundance in the top layers of raw material mound $h = 1.0$ m; 2 – CFU abundance in the bottom layers of raw material mound $h = 0.20$ m; 3 – the raw material moisture content of the top layers of the mound $h = 1.0$ m; 4 – GRMF moisture content in the bottom layers of the mound $h = 0.20$ m.
$w_0 = 23.2 \pm 0.3\%$; $M_0 = 9.2 \times 10^3 \pm 2.8 \times 10^3$ CFU·g^{-1}; $v_f = 0.24 \pm 0.05$ m·s^{-1}; $C_0 = 500$ ppb = 1070 μg·m^3; $C_0 = 1250$ ppb = 2675 μg·m^3

According to these results, it can be assumed that ozone negligibly influences the drying processes of food raw material, but it positively influences the reduction of mycobiotic contamination, especially at 1250 ppb ozone concentration (Fig. 6, c). 500 ppb ozone concentration was inadequate, and at the beginning of ventilation, until ozone had not penetrated through the whole GRMF mound, in the top layers the mycobiotic contamination was increasing, while in the bottom layers – changed insignificantly (Fig. 6, b). Ventilation with the air more saturated with ozone ($C_0 = 1250$ ppb) resulted in quicker ozonation of the top layers of the mound. Ozone penetration in the raw material mound depended on the initial concentration of ozone. While ozonizing, the GRMF was disinfected; its drying was somewhat induced; conditions for the micromycete activity were limited, therefore mycobiotic contamination of the top layer decreased by about 50% and of the bottom layer – three times (Fig 6, c).

Studies have shown that the abundance of micromycete CFU in non-ventilated GRMF during the first 2 days did not change significantly (increased by 20%), but after 4 days it was 1.5 times higher than the initial, and after 8 days – 2.5 times higher (Fig 6, d). No differences in mycobiotic contamination between the top and bottom layers of the GRMF were observed. In the GRMF untreated with ozone the micromycete species diversity was higher: 26 species were recorded in non-ventilated raw material and only 11 species in the ventilated GRMF.

The GRMF drying with active ventilation supplied with ozonized air can not only halt the development of micromycetes but also reduce their abundance and change their species composition in the raw material. Micromycetes of the *Fusarium* genus ceased their active functioning in the ozonized GRMF. It is assessed [61, 62, 63, 64] that micromycetes of the *Fusarium* and *Alternaria* genera mostly damage cereals still in the field. During harvesting, the wheat grain contamination by *Alternaria alternata* micromycetes often reaches 100%, but, after drying by active ventilation with the ozone-air mixture, the amount of these micromycetes significantly reduces [62]. The development of *Fusarium* micromycetes is strongly inhibited not only by the GRMF drying but also the used harvesting technologies.

It is stated that as a consequence of the ubiquitous existence of *Fusarium* spores in soil, the cutting height of the harvester machine will be an important factor for reducing the contact of soil with healthy grains [65, 66]. The fan speed of the

combine harvester must also be settled to optimize a first cleaning step directly on the field [65].

Micromycetes of some *Aspergillus* species, which are resistant to external factors, often remain active in the GRMF. One of the main reasons of their survival is the sufficient moisture content in the raw material mound. It has been determined [65] that *Aspergillus* spp., which are the less demanding fungi, can grow at low water levels (13.5–18%), while *Fusarium* spp. need 170–190 g moisture·kg^{-1} substrates. After wheat ozonation, their moisture content was 14%, and *Aspergillus niger* as well as *A. clavatus* survived. According to our results and data obtained by other authors [28], micromycetes of the *Aspergillus* and *Cladosporium* genera can be considered the most resistant among all fungi recorded on the GRMF. Some researchers [67, 68, 69] indicate that one of the most effective measures in reducing the infections of *Aspergillus* micromycetes in the GRMF is the use of lactic acid bacteria. These bacteria are characterized by high antifungal activity and have beneficial health effects, but their use is restricted by the lack of efficient technologies and limited effects on some fungi. Mendez *et al.* [6] states that the impact of ozone supplied into the GRMF mound is particularly effective against some micromycetes of the *Aspergillus* genus. The authors point out that grain ozonation reduced the spread of *Aspergillus parasiticus* micromycetes by 63%, and they are known as active producers of mycotoxins of the aflatoxin group and thus quite hazardous to human health.

In the course of the study, the CFU abundance of mycotoxin-producing micromycetes in the GRMF affected by ozone-air mixture was reduced, but the CFU were not completely eliminated. Micromycetes capable to produce and release into the environment mycotoxins hazardous to human health – ochratoxin, patulin, cytochalazin, moniliformin, mycoine – mostly survived.

Ozone is a reliable measure reducing the mycobiotic contamination of food stock produced from cereal grains, helping to avoid the risk to human health caused by toxic secondary metabolites synthesized by micromycetes and released into the environment. However, it is essential to further improve the efficiency of ozone application technologies, expand the spheres of its use in order to maintain food free from mycobiotic contamination as long as possible and to improve the food safety and people's health conditions.

GRMF treatment with electrochemically activated water (anolyte). Performed using 3 different chlorine concentrations: 0.01, 0.02 and 0.05%. Chlorine is one of the basics ingredients in anolyte solution that acts on microorganisms [41, 70]. Huang [70] *et al.* reported that concentration of chlorine and proper its application is very important factors for inactivating of micromycetes. They defined that free chlorine of anolyte, mainly hypochlorous acid (HOCl), produces hydroxyl radical that acts on microorganisms. Anolyte is produced by passing a diluted salt solution through an electrolytic cell. Therefore the greatest advantage of anolyte for the inactivation of pathogenic microorganisms relies on its less adverse impact on the environment as well as users' health because of no hazard chemicals added in its production.

It has been determined that an inhibitory effect against micromycete propagules present on the surface of the GRMF was obtained only when the treatment with 0.05% concentration of chlorine solution was applied (Fig. 7).

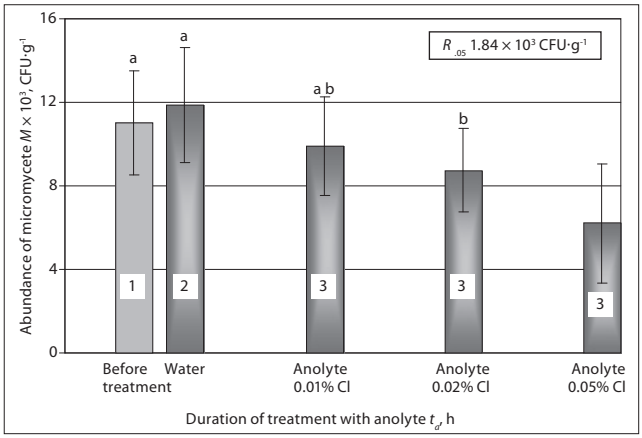

Figure 7. Effect of anolyte concentration on mycobiotic contamination of wheat raw material: 1 – initial abundance of CFU in raw material, 2 – abundance of CFU in raw material after 12 hours storage in H_2O, 3 –abundance of CFU in raw material after 12 hours impact of anolyte ($M_0 = 1.1 \times 10^4 \pm 2.5 \times 10^3$ CFU·g^{-1}).
[a, b] no statistically significant difference at the 95% confidence probability level was recorded between the mean values of the columns marked with the same letter.

Exposure to anolyte with lower chlorine concentrations was negligible for the GRMF mycobiotic contamination. Anolyte may be used instead of water during the GRMF lying prior to grinding. In such case the mycobiotic contamination of raw materials could be reduced almost 2 times.

It has been determined that anolyte is most effective during the period of 2 hours (Fig. 8). Further GRMF storage in anolyte solution had almost no impact on the reduction of mycobiotic contamination. Therefore, the optimal duration of raw material processing with anolyte is 0.5 hours.

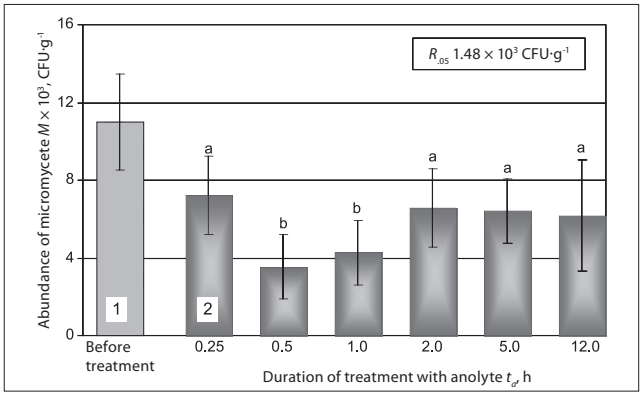

Figure 8. Influence of anolyte treatment duration t_d on mycobiotic contamination of wheat raw material: 1 – initial abundance of CFU in raw material, 2 – abundance of CFU in raw material after treatment with anolyte of 0.05% chlorine concentration ($M_0 = 1.1 \times 10^4 \pm 2.5 \times 10^3$ CFU·g^{-1}).
[a, b] no statistically significant difference at the 95% confidence probability level was recorded between the mean values of the columns marked with the same letter.

The investigations of the impact of anolyte on the development of different micromycete species revealed that anolyte of 0.05% chlorine concentration stopped the development and functioning of *Fusarium* and *Penicillium* fungi, while micromycetes of certain *Aspergillus* species remained viable.

CONCLUSIONS

Considering the fact that mycobiotic contamination of the grain raw material for food (GRMF) reduces its value and constitutes a hazard to human health, further search

for safety measures remains highly relevant scientific and technological problem, which can be successfully addressed only combining the forces of the researchers of various fields.

Recently a variety of measures are employed for the GRMF protection from mycobiotic contamination; active ventilation, electro-activated air (ozone) and electro-activated water (anolyte) take a significant place among them; these measures could inactivate vital functions of microorganisms and reduce their degradation and mycobiotic potential.

Mycobiotic contamination of the GRMF can be reduced by ventilating the raw material with optimum air flow at the air filtration velocity of 0.24 $m \cdot s^{-1}$.

Mycobiotic contamination of the GRMF can also be reduced using the anolyte treatment prior to grinding. Anolyte (0.05% chlorine concentration) could reduce the contamination by almost 2.5 times. The determined optimal processing time – 0.5 to 1 hour. The impact of anolyte is particularly effective against micromycetes of the *Fusarium* and *Penicillium* genus.

When drying the GRMF by active ventilation using the ozone-air mixture, ozone (O_3 concentration 1250 ppb) disinfects the raw material surface thus creating unfavourable conditions for increasing mycobiotic contamination in the top layers of the grain mound, which are the last to dry. Contamination is reduced by 50%, and in the bottom layers – for more than 3 times.

The ozone-air mixture significantly suppresses *Fusarium avenaceum*, *F. graminearum*, *F. poae*, *F. solani*, *F. tricinctum*, *F. sporotrichioides* micromycetes as well as the development of *Alternaria alternata* and other fungi in the GRMF. Micromycetes of various *Aspergillus* genus species are more likely to survive in the ventilated raw material.

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