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THE INFLUENCE OF DIFFERENT SPECIES OF YEAST ON MYCOTOXINS CONCENTRATIONS IN WHEAT

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The aim of this study was to evaluate the effect of *Saccharomyces cerevisiae*, *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast cell suspensions and *Saccharomyces cerevisiae* cell wall's polysaccharides and fermentation time on mycotoxins concentrations in feed wheat. The 2018 harvest three feed wheat samples were taken from grain processing companies, and the research was carried out at the Lithuanian University of Health Sciences, Veterinary Academy, Mycotoxicology Laboratory. The Thin-layer chromatography technique (TLC) was used to determine mycotoxins concentrations in the samples. The wheat samples were inoculated with *Saccharomyces cerevisiae*, *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast 10^4 cells ml⁻¹ suspensions and *Saccharomyces cerevisiae* cell wall's polysaccharides, duration of the wheat fermentation with the yeast were – 30 and 60 min. It was found that all yeast species suspensions had reducing effect of mycotoxins concentrations which correlated with longer duration of fermentation. *Saccharomyces cerevisiae* yeast suspension after 60 min reduced AFB₁ and DON by 100 % (p<0.05) and ZEA up to 80.1 ± 0.50 % (p<0.05). *Saccharomyces cerevisiae* yeast cell wall's polysaccharides decreased the concentrations of AFB₁ (p>0.05), ZEA (p<0.05), DON (p<0.05) during the experiment better than *Saccharomyces cerevisiae* 10^4 cells ml⁻¹ suspension and after 60 min exposure polysaccharides absorbed all wheat mycotoxins by 100 % (p<0.05). The AFB₁ was best absorbed by *Kluyveromyces marxianus* yeast after 60 min. of fermentation by 100 % (p<0.05). The DON concentration was below the detection limit after 60 min. of fermentation with *Geotrichum fermentans* and *Rhodotorula rubra* yeast.

Keywords: fermentation, mycotoxins, wheat, yeast, yeast cell wall's polysaccharides

INTRODUCTION

Wheat is one of the primary grain culture cultivated in Lithuania and the world. According to United Nations Food and Agriculture Organization statistics, world food and feed production use 141 to 510 million tonnes of wheat per year (FAO Food outlook..., 2019).

The quality of wheat is mostly affected by contamination by *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. mould fungi and their secondary metabolites – mycotoxins (Beukes et al., 2018). Aflatoxin B₁ (AFB₁), deoxynivalenol (DON), zearalenone (ZEA) and T-2 toxin - are dominant mycotoxins in wheat and their products (Mankevičienė et al., 2011; Asghar et al., 2016). Mycotoxins which are found in wheat easily can pass to animals feed and people food (Bol et al., 2016; Stanciu et al., 2017). Mycotoxins in feed can cause growth disturbances in the animal's body, reduces the body's growth capacity, nutrient uptake, cause changes in genetic systems – disruption of gene activity associated with the transport of nutrients, possible cellular oxidative damage (Wu et al., 2015).

After evaluating the contamination of feed wheat by mycotoxins, biological detoxification is increasingly used. Yeast can be chosen for this purpose because the morphological structure of the polysaccharides and especially, the β-D – glucans, which are found in the yeast cell wall's, have mycotoxins absorbing effect (Aazami et al., 2018). In addition, to the food *Saccharomyces cerevisiae* yeast used in the food and agriculture industry, other species may also reduce mycotoxins in wheat, for example such as – *Geotrichum fermentans*, *Rhodotorula rubra*, and *Kluyveromyces marxianus* (Repečkienė et al., 2013).

The aim of this study was to evaluate the effect of *Saccharomyces cerevisiae*, *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast cell suspensions and *Saccharomyces cerevisiae* cell wall's polysaccharides and fermentation time on mycotoxins concentrations in feed wheat.

MATERIAL AND METHODS

In 2018 during September – December, 2018 year harvest wheat samples (three replicates) were collected from grains processing companies. In the Mycotoxicology Laboratory of Veterinary Academy of Lithuanian University of Health Sciences has carried out mycotoxins analysis and biological detoxification of wheat with food yeast *Saccharomyces cerevisiae* and their cell wall's polysaccharides and also with *Geotrichum fermentans*, *Rhodotorula rubra*, *Kluyveromyces marxianus* yeast, which was extracted from various vegetable substrates.

Determination of mycotoxins concentrations. In wheat samples AFB1, ZEA concentrations were analyzed by Thin-layer chromatography (TLC) and described by Romer Labs Inc. ®Method (Austria) (Code: a/z-tl-01-00.2) and deoxynivalenol (DON) (Code: CAM-000031-1). For AFB1 and ZEA concentrations, silica gel (SG) plates were air-dried, and the results were evaluated on a ultraviolet (UV) background (360 nm). For DON concentration determination the SG plate was heated at 150°C for 5 min and 360 nm UV background was chosen. The detection limit of DON and ZEA is 10 µg/kg and AFB1 – 1 µg/kg (Razzazi-Fazeli et al., 2002).

Yeast suspension production and inoculation of wheat. The yeast culture of *Saccharomyces cerevisiae* obtained from pressed baking yeast, while *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast were extracted from various vegetable substrates (corn, vegetables, fruits) and confirmed by PGR in the Institute of Botany of Nature Research centre, Biodeterioration Research Laboratory, Vilnius, Lithuania. Yeast cultures were multiplied by dilutions up to 10⁻⁶ and inoculated on Sabouraud Dextrose Agar (OXOID, UK) (SDA) surface. After 7 days of incubation in a thermostat at 28 ± 2°C, the culture inoculated on a SDA following Samson et al. (1988) and incubated for 4 days at 28 ± 2°C. For inoculum production, the yeast cell density is calculated using a Neubauer improved bright-line hemocytometer (Germany) of 0.100 mm (0.0025 mm²). Yeast cells counted by microscope (Motic BA 310E, Germany) using 40 × magnification and prepared a 10⁴ cells ml⁻¹ suspension *in vitro*, three feed wheat samples were inoculated based on Bakutis et al. (2005) modified method. During the research wheat samples (15 g) were grounded, moistened with 3 ml of sterile water and treated with 1.5 ml of yeast (respectively *Saccharomyces cerevisiae*, *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus*) 10⁴ cells ml⁻¹ suspensions. The fermentation was carried out at 28 ± 2°C for 30 and 60 min. Experiments were repeated three times.

Isolation of polysaccharides from the cell walls of *Saccharomyces cerevisiae*. The *Saccharomyces cerevisiae* yeast cell walls were destroyed based on Dallies et al. (1998) modified methods. For the isolation of polysaccharides, 20 mg wet weight of *Saccharomyces cerevisiae* was mixed with 0.5 ml 2°C Tris hydrochloride (Tris-HCl) (10 mM, pH 8). Sterile glass beads (0.5g, 0.45 – 0.55 mm in diameter) were used to break down the yeast cell wall's. Test-tube in which were *Saccharomyces cerevisiae* yeast with Tris-HCl buffer and glass beads were shaken in a V-1 Plus shaker with ice (BioSan, Latvia) 3000 rpm at 4 times 20 following seconds, break time - 1 min. After 4 shaking intervals, the percentage of cell walls destroy is assessed by microscopy using a 0.100 mm (0.0025 mm²) Neubauer improved bright-line hemocytometer. The reconstituted suspension was transferred to a sterile tube, then the glass beads were washed with 1 ml Tris-HCl buffer solution cooled to 2°C, and the resulting liquid phase was suction from the beads and placed in a standard tube, which is prepared for centrifugation for 5 min 1500 rpm (Eppendorf 5810R, Germany). The only transparent surface liquid was used for the research (Dallies et al., 1998). Polysaccharide treatment of wheat samples were analogous to suspension processing. The experiment repeated three times.

Statistical analyses. The data analysed by SPSS statistical package (SPSS for Windows 17.0, SPSS Inc., Chicago, IL, USA, 2008) by 1-way analysis of variance (ANOVA). The results were considered to be reliable under P < 0.05.

RESULTS

The results of this study indicate that *Saccharomyces cerevisiae* yeast and their cell wall's polysaccharides and from various vegetable substrates extracted *Geotrichum fermentum*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast have reducing effect to the mycotoxins. The TLC technique was used to determine mycotoxins concentrations in the feed wheat samples before the experiment: in the first wheat sample was detected – 670 µg/kg of ZEA and 500 µg/kg of DON; in the second sample – 250 µg/kg of DON; in the third – 7 µg/kg of AFB1 and 700 µg/kg of ZEA.

The results of averages (three replicates) of feed wheat samples inoculation with *Saccharomyces cerevisiae* yeast suspension and *Saccharomyces cerevisiae* cell wall's polysaccharide with different fermentation time ar submitted in Figure 1. *Saccharomyces cerevisiae* yeast suspension after 30 min of fermentation reduced AFB1 by 67.1% (p>0.05) and DON – 57 % (p>0.05), while *Saccharomyces cerevisiae* yeast cell wall's polysaccharides AFB1 decreased by 77.1% (p>0.05) and DON – 100 % (p<0.05). *Saccharomyces cerevisiae* yeast suspension and *Saccharomyces cerevisiae* yeast cell wall's polysaccharides both after 30 min of fermentation decreased ZEA by 54.4 % (p>0.05) and after 60 min of fermentation reduced ZEA – 100 % (p<0.05).

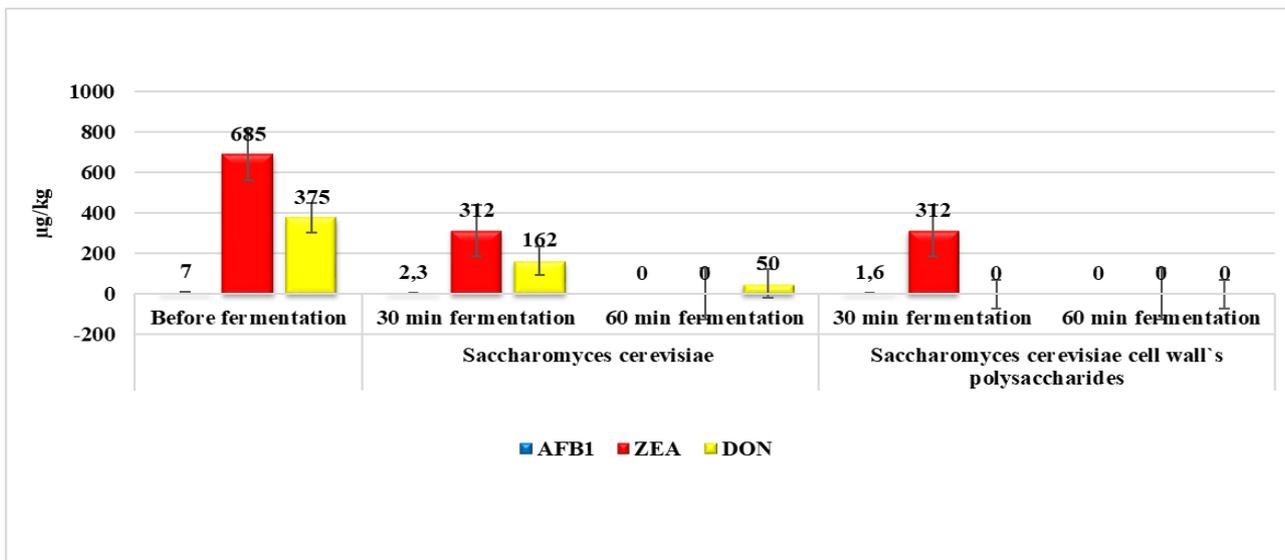


Figure 1. Feed wheat inoculation with *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae* cell wall's polysaccharide

Saccharomyces cerevisiae yeast suspension after 60 min reduced AFB1 and ZEA by 100 % ($p < 0.05$) and DON up to 87 % ($p < 0.05$). *Saccharomyces cerevisiae* yeast cell wall's polysaccharides decreased the concentrations of AFB1 ($p > 0.05$), DON ($p < 0.05$) and ZEA ($p < 0.05$) during the test better than *Saccharomyces cerevisiae* yeast suspension, and after 60 min exposure polysaccharides reduced all mycotoxins by 100 % ($p < 0.05$).

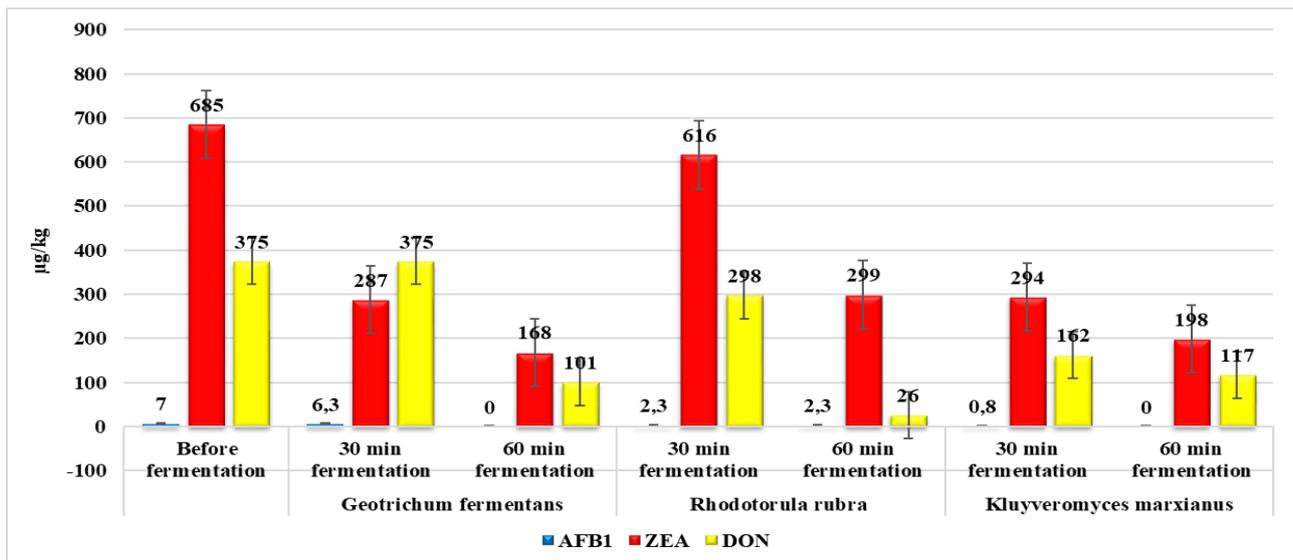


Figure 2. Feed wheat inoculation with *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast

The results of averages (three replicates) of feed wheat samples inoculation with *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast suspensions with different fermentation time are submitted in Figure 2. After 60 min of fermentation AFB1 concentration in feed wheat samples significantly reduced by *Geotrichum fermentans* and *Kluyveromyces marxianus* yeast cell suspensions – AFB1 concentration was below detection limit ($p < 0.05$).

Geotrichum fermentans and *Kluyveromyces marxianus* yeast cell suspension significantly decreased ZEA concentration in feed wheat samples after 60 min of fermentation. *Geotrichum fermentans* yeast suspension ZEA concentration reduced by 75.4 % ($p > 0.05$) and *Kluyveromyces marxianus* – 71.1 % ($p > 0.05$).

DON concentration after 60 min of feed wheat fermentation with *Rhodotorula rubra* yeast cell suspension decreased by 93.1 % ($p < 0.05$).

CONCLUSIONS AND DISCUSSION

Our data indicate that *Saccharomyces cerevisiae* yeast 10^4 cell ml^{-1} suspension, a mixture of these yeast cell wall's polysaccharides and *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast suspensions had reducing effect on the mycotoxins concentrations in feed wheat.

Comparing *Saccharomyces cerevisiae* yeast 10^4 cell ml^{-1} suspension and cell wall's polysaccharides was observed, that cell wall's polysaccharides showed the strongest reducing effect on the mycotoxins concentrations. Within 60 min

fermentation 1.5 ml polysaccharides 100% reduced concentrations of AFB₁, DON, and ZEA. It can be assumed that these differences are caused by glycoproteins and lipids removed from the yeast cell walls. In addition, our study found that 60 min wheat exposure to *Saccharomyces cerevisiae* polysaccharides was more effective than 30 min. of fermentation, and similar dependence was also observed by Aazami et al. (2018). In the above-mentioned study, it was found that purified polysaccharide reduces AFB₁ concentrations more than 2 fold than the untreated cell wall's of *Saccharomyces cerevisiae* and based on the results of our study, it has been found that *Saccharomyces cerevisiae* cell wall polysaccharides efficacy were 1.4 times higher.

Also, Repečkienė et al. (2013) analysed the use of yeast 10⁴ cells ml⁻¹ suspension for mycotoxins concentrations reduction. Comparing the results of their and our research, the most pronounced difference is observed in the decrease of ZEA concentration. In our study, the effect of *Saccharomyces cerevisiae* yeast suspension (after 60 min of fermentation), ZEA on average decreased by 69 % (p>0.05) in wheat samples, and they found that yeast reduced the ZEA by 87 %, but the effect of fermentation duration has not been analysed.

During the experiment also has been observed from various vegetable substrates extracted *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast effect on mycotoxins concentrations. According to scientific literature, from various vegetable substrates isolated yeast the efficiency of mycotoxins detoxification is uneven, which may be due to morphological features of the yeast cell wall, enzyme systems activity and interactions with mycotoxins, leading to metabolic changes (Jakopovic et al., 2018). Repečkienė et al. (2013) have found that *Geotrichum fermentans* and *Kluyveromyces marxianus* reduces aflatoxins by 100 %, and in our study, after 60 min duration of fermentation was also observed at 100 % (p<0.05) absorption of AFB₁ concentration with this yeast. However, the results are different in ZEA and DON concentrations. In our study it was found that after 60 min of fermentation ZEA concentration with *Kluyveromyces marxianus* and *Geotrichum fermentans* yeast decreased up to 81% (p<0.05) and the study said that after this time yeast activity the ZEA concentration is below the detection limit. Also, comparing the effect on DON concentration, Repečkienė et al. (2013) found that 7% more DON is absorbed by *Kluyveromyces marxianus* than *Geotrichum fermentans* and our study found that DON was more effectively reduced by the yeast of *Geotrichum fermentans*. Based on the results of the mycotoxicological study, *Rhodotorula rubra* yeast is useful for reducing the concentration of DON after 60 min. of fermentation, as absorption is 90 - 100%.

Fermentation time is a significant factor in feed wheat inoculation with yeast suspensions and cell wall's polysaccharides, to reduce the amount of mycotoxins. The best results were found after 60 min feed wheat fermentation with *Saccharomyces cerevisiae*, *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast suspensions and *Saccharomyces cerevisiae* yeast cell wall's polysaccharides. Our study research shows that after 60 min of feed wheat fermentation with *Saccharomyces cerevisiae* yeast suspension AFB₁, DON and ZEA concentrations decreased better than after 30 min of fermentation. It was found that the best AFB₁ and ZEA reducing effect in feed wheat was after 60 min of fermentation with *Geotrichum fermentans* and *Kluyveromyces marxianus* yeast cells suspensions. DON concentration best reduced *Rhodotorula rubra* yeast cells suspension in feed wheat samples after 60 min of fermentation.

Saccharomyces cerevisiae cell wall's polysaccharides reduced AFB₁ (p>0.05), DON (p<0.05) and ZEA (p<0.05) mycotoxins concentrations by 100 % (p<0.05) in feed wheat after 60 min of fermentation and it was more effectively than the *Saccharomyces cerevisiae* yeast 10⁴ cells ml⁻¹ suspension.

Based on the results of the obtained research studies it was found that *Saccharomyces cerevisiae* yeast 10⁴ cells ml⁻¹ suspension, *Saccharomyces cerevisiae* cell wall's polysaccharides and from the various vegetable substrates extracted *Geotrichum fermentans*, *Rhodotorula rubra* and *Kluyveromyces marxianus* yeast suspensions can be used to reduce mycotoxins in feed wheat, after more detailed studies with fermentation duration, different yeast species and cellular concentrations.

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