Yeast cell walls adsorption capacity

V V Solovyov\(^1\), A M Marhunova\(^1\), O L Permiakova \(^1\), T V Voblikova \(^2\) and Yu O Semenova \(^2\)

\(^1\)RUE “Scientific and Practical Center for foodstuff of the National Academy of Sciences of Belarus”, Republic of Belarus, Minsk, Kozlova str., 29
\(^2\)Yaroslav-the-Wise Novgorod State University, 41, ul. B. St. Petersburgskaya, Veliky Novgorod, Russian Federation

E-mail: tppshp@mail.ru

Abstract. The article considers the problem of effective and safe ways to disinfect feed from mycotoxins using the adsorption capacity of yeast cell walls. We present the results of the study on yeast cell walls physicochemical properties and adsorption capacity by high performance liquid chromatography, using a reference sample of Trylogy multitoxins, in relation to such mycotoxins as aflatoxin B1, ochratoxin A, zearalenone, deoxynivalenol. The obtained results show that yeast cell walls have good adsorption capacity with respect to heavy mycotoxins and poorly absorb to light mycotoxins.

1. Introduction

Mycotoxin contamination of feed is a global problem. As a result of the analysis carried out in June-August 2014, it was found that 97.5% of grain samples taken worldwide contain two or more mycotoxins, and their average amount was 6.7 per sample. Trichothecenes of type B, fusargic acid and fumonisins, found in 71% of samples, were the most common. The simultaneous presence of several of them increases the toxic effects on animals, which leads to a much greater deterioration in health and productivity than would be expected. At the same time, a variety of symptoms are observed - depending on the toxins type and concentration, the livestock type and age, as well as its productivity. A high content of toxic substances causes the rapid development of pronounced clinical signs, and a low content can have a slow effect on productivity [1].

Mycotoxins disrupt the internal organs functioning, including the gastrointestinal tract, nervous, endocrine, immune and reproductive systems. All this leads to a decrease in the growth or a deterioration in feed conversion, a drop in milk production, the occurrence of diseases [2–4].

In accordance with the Veterinary and Sanitary Rules for ensuring the safety of feed, feed supplements and raw materials for compound feeds production, in grain supplied for feed (wheat, barley, oats, rye, triticale, millet, sorghum, corn), the content of mycotoxins is regulated. The safety requirements for mycotoxins permissible content in grain are presented in table 1.

| Name of mycotoxin | Mycotoxin content, mg / kg, no more than |
|-------------------|-----------------------------------------|
| Aflatoxin B\(_1\) | 0.02                                    |
| Ochratoxin A      | 0.05                                    |
| T-2 toxin         | 0.1 |
|------------------|-----|
| Deoxynivalenol (DON) | 1.0 |
| Zearalenone       | 1.0 |
| Fumonisin         | 5.0 - (corn) |
| Sum of aflatoxins B₁, B₂, G₁, G₂ | 0.02 |

The yeast used in brewing belongs to the class Ascomycetes, order Endomycetales, family Saccharomycetaceae, genus Saccharomyces, species Saccharomyces cerevisiae and Saccharomyces carlsbergensis. Yeast Saccharomyces carlsbergensis perform bottom-fermented beer wort. This yeast is used to prepare standard and varietal beers. Yeast Saccharomyces cerevisiae are top fermented yeast and are used mainly for dark and special sorts of beer.

During the analysis of literary sources, it was found that the yeast cell consists on average of 75% of water and 25% of dry substances. Their chemical composition varies depending on race, nutrient medium and physiological state.

The dry matter composition varies within the limits, namely:
- protein substances – from 40 to 60%;
- carbohydrates - from 25 to 35%;
- fats (lipids) - from 4 to 7%;
- minerals - from 6 to 9%.

90% of the total amount of yeast protein goes to true proteins. The best known of these are zimo-casein (phosphoprotein) and cerevisin (albumin). The content of nucleoproteins, which the nuclei of yeast cells are rich in, is 26% of the total amount of proteins. Nucleoprotein acids (a prosthetic group of nucleoproteins) upon hydrolysis form purine and pyrimidine bases, sugar (ribose or deoxyribose) and phosphoric acid [4].

In recent years, β-glucans attract close attention as immunomodulators – substances with onco- and radioprotective properties. Beta-glucans of yeast and fungi, in contrast to grain glucans, have a branched structure.

Yeast cell walls have a layered structure. The outer layer of the cell wall is a smooth membrane, underneath is a amann-protein complex. The inner layer contains amorphous mannans, structure forming β-glucans and proteins linked by a network of microfibrils consisting of glucans. The main structural component of the yeast cell wall is polysaccharide. The content of polysaccharides in the cell wall of Saccharomyces cerevisiae can be up to 90% of the cell wall mass. Chemical analysis of the cell wall shows that it consists mainly of glucan and mannan; along with these components, chitin and protein are present in the wall. Glucan is a complex branched glucose polymer located in the inner layer of the cell wall adjacent to the plasma membrane. Apparently, glucan is the main structural component of the cell wall, since during its removal it is completely destroyed. Mannan (a complex polymer of mannose) is found in the outer layers of the cell wall area. Removal of mannan does not change the overall cell shape, so it is obviously not essential to maintain the cell wall integrity. The third carbohydrate component of the wall, chitin, is a polymer of N acetylglucosamine; it is found in the Saccharomyces cerevisiae cell wall areas associated with daughter scars. Protein makes up 10% of the cell wall dry mass. At least part of this protein is in the form of wall-bound enzymes. Some of these enzymes (for example, invertase) are mannanproteins and contain up to 50% of mannan as a molecule constituent. Many of the rest of the cell wall proteins are also associated with mannan, so the latter, apparently, performs a structural function in the cell wall [5].

The cell wall of Saccharomyces cerevisiae contains amino sugars of the glucosamine type, which are not similar to chitin and lychitosan. These amino sugars, as a rule, are an integral part of glycoproteins. The cell wall consists of 40% mannanproteins and 2% chitin; the rest is β-glucan. The β-1,3 and β-1,6-glucans of the cell wall of Saccharomyces cerevisiae are one polymer with a molecular mass ~ 240000, in which the main chain is represented by β-1,6-glucan, and the side chains are represented by β-1,3-glucoside residues.
In the yeast cell walls, β-glucan is located in the inner layer, where it is linked to protein, mannan, and chitin. The biological effect of β-glucans is determined by many factors: the type and configuration of bonds between monosaccharide residues, the macromolecule branching and conformation, its polymerization degree; solubility in water, etc. It is found that soluble β-glucans have greater physiological activity than insoluble ones. It is shown that, in comparison with other glucans, Saccharomyces cerevisiae yeast β-(1→3), (1→6)-D-glucan exhibits the highest physiological activity [6–10].

As a result of the analysis of literary sources, it was noted that the polysaccharide shell of yeast cells possesses a high adsorption capacity for high molecular weight mycotoxins (Oxratoxin A, T-2 toxin, Deoxynivalenol (DON), Zearalenone) and practically do not adsorb low molecular weight mycotoxins (Aflatoxin B1, Fumonizin).

The functional properties of the mycotoxin adsorbent are as follows:
– can adsorb a significant amount of bile acids, toxins and electrolytes, can remove heavy metal ions and radionuclides;
– have a positive effect on the digestive tract microflora, have a bactericidal effect;
– the effect of the adsorbent is like a filter, due to its biocompatibility, it preserves important elements, sugars and amino acids, neutralizing the action of toxins and mutagens.

The use of yeast cell walls as an enterosorbent in feed has an indisputable advantage over inorganic enterosorbents, since its most characteristic is selectivity, while non-selective enterosorbents, removing part of useful substances from the chyme, can lead to complications, especially with long-term use.

To date, there are some sorbent preparations in the world based on yeast cell walls, and there is also data on their ability to adsorb mycotoxins.

The main sorbent for mycotoxins is “Mikosorb” (Alltech. USA), obtained on the basis of glucans isolated from the inner part of the yeast cell wall. A similar product is made by the Angel Company (China). “Mikosorb” possesses an adsorbing ability against a wide spectrum of mycotoxins.

"Mikosorb" effectively adsorbs mycotoxins in the gastrointestinal tract of agricultural animals and poultry. This fact is confirmed by Russian and foreign scientists. The optimum rate for poultry is from 500 to 1000 g per 1 ton of feed. The use of the adsorbent "Mikosorb" in the amount of 0.5 kg per ton of feed contributed to the elimination of symptomatic mycotoxicosis in broiler chickens, increased the safety and productivity of poultry. Mycotoxicosis was caused by natural contamination of feed with aflatoxin B1 (2-150 μg/kg of feed), zearalenone (50-600 μg/kg of feed), ochratoxin A (2-40 μg/kg of feed), T-2 toxine (25-250 μg/kg of feed), deoxyvalenol (30-1100 μg/kg of feed), sterigmatocystin (25-270 μg/kg of feed), as well as by unidentified mycotoxins found in the combined feed.

There is a dependence of using "Mycosorb" and a decrease in the synergistic mycotoxins interaction in chickens. Inclusion into the diet of corn naturally contaminated by with mycotoxins (33 μg/kg aflatoxin, 0.39 mg/kg fumonisin, 0.39 mg/kg T-2 toxin and 1.4 mg/kg deoxyvalenol) led to a decrease in bird productivity. Synergism was shown between mycotoxins of corn and aflatoxin, added to the diet at a dose of 3 mg/kg. The addition of aflatoxin to the feed led to a growth of the liver and heart. The spleen weight was also higher in birds consuming contaminated corn + aflatoxin. The addition of Mikosorb to the feed (1 kg/ton of feed) reduced the mortality rate of birds that consumed feed contaminated with aflatoxin. Mikosorb was able to minimize the negative effects of mycotoxins [11].

As can be seen from the literature data, yeast cell walls are a promising organic sorbent for the feed industry. In connection with this, an urgent line of research is the study of the yeast cells polysaccharide shells adsorption capacity to heavy and light mycotoxins using a Trylogy reference sample of multitoxins by high-performance liquid chromatography.

2. Objects and methods of research

The research was carried out on the basis of the Republican Control and Testing Complex of food products quality and safety “Scientific and Practical Center for foodstuff of the National Academy of
Sciences of Belarus” on the calibrated equipment, providing the measurement results reliability, in the number of three parallel measurements.

The object of the study was the cell walls of hydrolyzed yeast.

To carry out analytical studies, the generally accepted organoleptic and physicochemical methods of analysis were used.

- Determination of pH was carried out using a pH-meter according to GOST 26188-84.
- Determination of dry substances content – by refractometric method according to GOST28562-90.
- Aflatoxin B₁ content according to GOST 31748-2012 (ISO 16050:2003).
- Ochratoxin A content was determined by MUK 4.1.2204-07.
- Zeaalenone content was also determined according to GOST 31691-2012.
- The content of deoxynivalenol (DON) was determined according to STB GOST R 51116-2002.

To obtain yeast cell walls, directed hydrolysis of yeast Saccharomyces carlsbergensis cells biopolymers was carried out according to previously established optimal modes and parameters.

To carry out the experimental work, we used enzyme preparations, the characteristics of which are presented in table 2.

**Table 2.** Characteristics of enzyme preparations used in yeast cell biopolymers hydrolysis.

| Name of the enzyme preparation | Standardized activity |
|-------------------------------|-----------------------|
| Thermostable α-amylase (Liquaflo) | 1460 units AC/cm³ |
| Acid protease (Talzim AP-50) | 420 units PC/cm³ |
| Glucoamylase (Sakhzyme Plus 2X) | 22300 units GLC/cm³ |
| Lipase | 45000 units LC/cm³ |
| Mannanase | 22000 units MC/g |

The targeted hydrolysis of yeast cell biopolymers was carried out in a laboratory fermenter, and the yeast biomass in the volume 10 dm³ was preliminarily acidified with lactic acid to pH from 5.2 to 5.4, and hydrolysis was carried out with intensive stirring of the hydrolyzed medium. Before hydrolysis, a preliminary inactivation of endogenous enzymes in yeast was carried out by increasing the temperature of the yeast biomass to 95 °C. In the beer yeast suspension, the dry matter content determined by the refractometric method was 15.3%.

The enzyme preparations were added at the beginning of the hydrolysis process. The hydrolysis process parameters are presented in table 3.

**Table 3.** Yeast cell biopolymers hydrolysis parameters.

| Parameter value | Enzyme preparations | Dosage of yeast, unit of activity/g | pH | Pause |
|-----------------|---------------------|-----------------------------------|-----|-------|
| Name            |                     | t, °C | Time, min |
| Thermostable α-amylase | 1.0 | 38 | 50 |
| Acid protease   | 2.0 | 52 | 50 |
| Glucoamylase    | 10.0 | 5.3 | 65 | 40 |
| Lipase          | 2.0 | 77 | 50 |
| Mannanase       | 10.0 | 95 | 5 |

Hydrolyzed yeast suspension is a turbid, highly dispersed system consisting of fine particles and colloidal substances. The yeast cell walls separation from the liquid fraction was carried out on a laboratory unit (figure 1), consisting of a separator GEA type FTC 1-06-107, screw pump Burun C03/4-0.55/4, and a control panel.
Figure 1. Scheme of laboratory unit for hydrolyzed yeast separation.

The excess beer yeast hydrolyzate is a liquid substance consisting of autolysis and hydrolysis products of yeast cell biopolymers (amino acids, vitamins, low molecular weight carbohydrates, etc.) with a dry matter content of 10 to 20%. The hydrolyzate viscosity is at the level of milk viscosity and ranges from 1.72 to 2.25 mPa*s, depending on the yeast used and the temperature.

The yeast cell walls drying was carried out on an experimental spray dryer under laboratory conditions in the Institute of Meat and Dairy Industry. A schematic diagram of the experimental spray dryer is shown in figure 2.

When products are dried on spray dryers, there is a direct interaction between the product sprayed onto small particles and the heat-carrying agent (hot air). Due to the large contact area of the liquid product and the heat-carrying agent, a high drying rate is achieved, which makes it possible to obtain a high quality readily soluble powder. In addition, drying proceeds very quickly (from tenths to several seconds) and therefore even heat-sensitive materials do not have time to decompose.

As a result of the experimental work, it was found that the cell walls of yeast, in terms of their physicochemical and colloidal properties, are suitable for drying on spray dryers.

As a result of the studies, the following modes of drying the yeast cell walls on spray dryers were determined. Air temperature at the drying chamber entrance was from 175 to 185 °C, at the drying chamber outlet – from 70 to 80 °C; dry substances mass fraction in the mixture supplied to the dryer – 15 ± 5%; temperature of the mixture entering the dryer – from 15 to 20 °C.
1 – air inlet; 2 – electric air heater; 3 – spray nozzles; 4 – drying chamber; 5 – cyclone for separating product particles from a gas stream; 6 – dry product collector; 7 – bag filter; 8 – fan

**Figure 2.** Spray dryer scheme.

The experimental spray dryer of RUE “Institute of the Meat and Dairy Industry” is shown in figure 3.

**Figure 3.** Experimental spray dryer.
3. Results and Discussion
As a result of the enzymatic hydrolysis, hydrolyzed yeast biomass separation and subsequent drying, dry yeast cell walls were obtained. In appearance, dry cell walls of hydrolyzed yeast are a heterogeneous dry mixture (powder) of grey color with a yeast aroma.

Next, we studied the physicochemical and technological characteristics of the yeast cell walls and determined their adsorption capacity.

As a result of a comprehensive assessment of dry cell walls of hydrolyzed yeast:

- their good solubility in cold water in various dosages is noted;
- their good homogenization characteristics when mixed with flour from various cereals are noted;
- the organoleptic characteristics are established;
- the content of dry matter and moisture is determined;
- the adsorption capacity for heavy and light mycotoxins is studied.

The organoleptic characteristics of the cell walls of hydrolyzed yeast are presented in table 4.

| Indicator name | Characteristics                           |
|----------------|------------------------------------------|
| Appearance     | Powder                                   |
| Color          | Light-grey to grey, with yellowish tint  |
| Scent          | Minor yeast                              |
| Taste          | Neutral, with a slight yeast flavor      |

In the dried cell walls of yeast, the moisture content and the content of dry substances were determined by drying to a constant weight. As a result of the study, it was found that the moisture content in the studied samples of dry cell walls of hydrolyzed yeast is from 8 to 10%.

To study the adsorption capacity of the cell walls of hydrolyzed yeast, we used a reference sample of multitoxins (reference material Trylogy). Trylogy's multi-toxin reference sample is corn flour naturally contaminated with mycotoxins.

The mycotoxin content of the Trilogy reference sample is shown in table 5.

| Mycotoxin name          | Detection threshold | Average concentration value | Units of measurement |
|-------------------------|---------------------|----------------------------|----------------------|
| Aflatoxin B1            | 1.0 μg/kg           | 12.3                       | μg/kg                |
| Aflatoxin B2            | 1.0 μg/kg           | 0.7                        | μg/kg                |
| Aflatoxin G1            | 1.0 μg/kg           | 0.7                        | μg/kg                |
| Aflatoxin G2            | 1.0 μg/kg           | Not detected               | μg/kg                |
| Fumonisin B1            | 0.1 mg/kg           | 6.0                        | mg/kg                |
| Fumonisin B2            | 0.1 mg/kg           | 1.9                        | mg/kg                |
| Fumonisin B3            | 0.1 mg/kg           | 0.6                        | mg/kg                |
| Ochratoxin A            | 1.0 μg/kg           | 8.6                        | μg/kg                |
| Zearalenone             | 50.0 μg/kg          | 330.9                      | μg/kg                |
| Deoxynivalenol (DON)    | 0.1 mg/kg           | 2.7                        | mg/kg                |
| T-2 toxin               | 5.0 μg/kg           | 332.2                      | μg/kg                |
| NT-2 toxin              | 5.0 μg/kg           | 240.1                      | μg/kg                |
To study the adsorption capacity of yeast cell walls, 4 samples were prepared basing on the Trylogy reference sample:

- sample 1 (control) - 50 g of reference sample of multitoxins + 150 ml of distilled water;
- sample 2 - 50 g of a reference sample of multitoxins + 0.05% dry cell walls of hydrolyzed yeast (0.025 g) + 150 ml of water;
- sample 3 - 50 g of a reference sample of multitoxins + 0.2% dry cell walls of hydrolyzed yeast (0.1 g) + 150 ml of water;
- sample 4 - 50 g of a reference sample of multitoxins + 0.35% dry cell walls of hydrolyzed yeast (0.175 g) + 150 ml of water.

All samples were exposed for 2 hours with periodic stirring in order to increase the mycotoxin extraction degree. Then the samples were subjected to phase separation on an ELMI CM-6M laboratory centrifuge for 6 minutes at a speed of 3500 rpm. The liquid phase was investigated by the content of mycotoxins in the method in high performance liquid chromatography in accordance with GOST.

The examination provided data on the content of aflatoxine B₁, ochratoxin A, zearalenone, deoxynivalenol in the studied samples, shown in figure 4.

![Figure 4. The result of the study of yeast cell walls adsorption capacity.](image)

The introduction of yeast cell walls into a medium contaminated with mycotoxins in an amount of 0.05% will reduce the content of ochratoxin A from 0.00213 to 0.00196 mg/kg, the content of
zearalenone from 0.061 to 0.059 mg/kg, the content of deoxyvalenol from 0.00327 mg/kg to 0.00292 mg/kg.

An increase in the dosage of the cell walls of hydrolyzed yeast into an environment contaminated with mycotoxins from 0.05% to 0.35% allows the content of ochratoxin A to be reduced from 0.00196 to 0.0012 mg/kg, the content of zearalenone from 0.059 to 0.054 mg/kg, the content of deoxynivalenol from 0.00292 mg/kg to 0.00209 mg/kg.

4. Conclusion
Taking into account the results obtained during the research, it was found that the cell walls of hydrolyzed yeast have a high adsorption capacity for high molecular weight mycotoxins at a dosage of 0.05% (Ochratoxin A, Zearalenone, Deoxyvalenol) and do not adsorb low molecular weight mycotoxin – Aflatoxin B1.

References
[1] 2017 Miragro.com - information and service portal of agriculture [Electronic source] http://miragro.com/obzor-rynka-neutralizatory-mikotoksinov-tingibitory-pleseni.html (accessed: 16.11.2017)
[2] Kuznetsov A F 2001 Veterinary mycology (SPb: “Lan’”) p 416
[3] Efimochkina N R, Sedova I B, Sheveleva S A and Tutelian V A 2019 Toxigenic properties of microscopic fungi Bulletin of Tomsk State University. Biology 6–33
[4] Tamkovich S N, Chelobanov B P and Duzhak T G 2015 Method of isolating nucleoprotein complexes circulating in the blood Bulletin of the Academy of Sciences. Chemistry 1458–1463
[5] Basha H and Ramanujam B 2015 Growth promotion effect of Pichia guilliermondii in chilli and biocontrol potential of Hansemiaspora uvarum against Colletotrichum capsici causing fruit rot Biocontrol Science & Technology 25 (2) 185–206
[6] Gematdinova V M, Kanarsky A V, Kanarskaya Z A and Bogdanov I V 2019 Obtaining β-glucan concentrate by germinating oats Chemistry of plant raw materials (Barnaul) 231–237
[7] Prasad R, Alok J, Latha S, Arvind K and Unnikrishnan V S 2015 Nutritional advantages of oats and opportunities for its processing as value added foods – a review J. Food Sci. Technol. 52 (2) 662–675 DOI: 10.1007/s13197-013-1072-1
[8] Sternaa V, Zutbee S and Brunavaa L 2016 Oat grain composition and its nutrition benefice Agriculture and Agricultural Science Procedia 8 252–256 DOI: 10.1016/j.asапр.2016.02.100
[9] Beloshapka A N, Buft P R, Fahey G C and Swanson K S 2016 Compositional Analysis of Whole Grains, Processed Grains, Grain Co-Products, and Other Carbohydrate Sources with Applicability to Pet Animal Nutrition J. Foods 5 1–16 DOI: 10.3390/foods5020023
[10] Nelson K, Stojanovska L, Vasiljevic T and Mathai M 2015 Germinated grains: a superior whole grain functional food Canadian Journal of Physiology and Pharmacology 91 (6) 429–441 DOI: 10.1139/cjpp-2012-0351
[11] Ovchinnikov A A and Tukhbatov I A 2017 Sorbent and probiotic, which is better? Materials of the international scientific and practical conference Ministry of Agriculture of the Russian Federation; Department of Science and Technology Policy and Education; South Ural State Agrarian University 173–179