Mgr inż. Krystian KLIMCZAK Dr inż. Monika CIOCH-SKONECZNY Department of Fermentation Technology and Microbiology, Faculty of Food Technology University of Agriculture in Krakow, Poland Katedra Technologii Fermentacji i Mikrobiologii, Wydział Technologii Żywności Uniwersytet Rolniczy w Krakowie, Polska

MYCOTOXINS IN BEER®

Mykotoksyny w piwie®

Beer is currently the most popular alcoholic beverage in the world. Due to the scale of consumption, ensuring maximum health safety of it is extremely important issue. One of it's safety risks is the possibility of mycotoxin occurrence. These compounds were first discovered in the 1960s, but new information about their properties is being discovered to this day. Mycotoxins are metabolites of cereal attacking mold fungi which can contribute to a wide range of conditions, from foodborne illnesses to various types of cancer. As contamination may occur at various leading to the production of a finished product,, manufacturer's awareness of this type of hazard is an important issue. The article discusses the most common groups of mycotoxins found in beer, brings up the issue of their origin and impact on the sensory characteristics of beer. In addition, article presents data on the occurrence of these compounds in beverages available on the market, as well as methods that can reduce their content.

Key words: fungi, mycotoxins, aflatoxin, *Fusarium*, beer.

Piwo jest obecnie najpopularniejszym napojem alkoholowym na świecie. Ze względu na skalę konsumpcji, bardzo ważną kwestią jest zapewnienie maksymalnego bezpieczeństwa zdrowotnego tego produktu. Jednym z zagrożeń jest możliwość występowania mykotoksyn. Związki z tej grupy zostały po raz pierwszy odkryte w latach 60 ubiegłego wieku, a nowe informacje dotyczące ich właściwości pojawiają się po dziś dzień. Są one metabolitami grzybów pleśniowych atakujących zboża i mogą przyczyniać się do występowania szerokiej gamy schorzeń, od zatruć pokarmowych aż do różnego rodzaju nowotworów. Skażenie może wystąpić na różnych etapach prowadzących do otrzymania gotowego produktu, stąd istotną kwestią jest świadomość producentów dotycząca zagrożenia. Artykuł omawia najczęściej występujące grupy mykotoksyn w piwie, porusza kwestię ich pochodzenia i wpływu na cechy sensoryczne produktu. Dodatkowo przedstawione zostały dane dotyczące występowania tych związków w piwach dostępnych na rynku, jak i metody mogące zmniejszyć ich zawartość.

Słowa kluczowe: grzyby, mykotoksyny, aflatoksyna, *Fusarium*, piwo.

INTRODUCTION

For years, beer has been widely appreciated among consumers. According to WHO [25] it is the most popular alcoholic beverage in Europe, with an average annual *per capita* consumption in the year 2018 ranging from 33 to 141 L, depending on country. For many years the highest consumption is observed in Czechia, where in 2018 the average annual consumption was 141 L. Subsequent countries in terms of consumption are Austria, Germany and Poland, respectively 107, 102 and 100 L *per capita* [23,25]. The size of this market can be exhibited by production volume, which only for European countries, reached 406 050 hL in 2018 alone.

Due to consumption volume, ensuring proper quality and health safety of the product is an extremely important issue. In case of toxic contamination, considerable and regular consumption which is observed in European countries, can bring health hazard to consumers. Toxic agents that may be present in beer include those presented in Table 1. In recent years, special attention began to be paid to mycotoxin occurrence in food products [13].

The aim of this article is to discuss issues regarding mycotoxin occurrence in beers. The article provides information on origination of these compounds in a product, their influence on sensory characteristics of beer and shows methods of decreasing their levels in a product.

Corresponding author–Adresdokorespondencji: Monika Cioch-Skoneczny, Katedra Technologii Fermentacji i Mikrobiologii, Wydział Technologii Żywności, Uniwersytet Rolniczy w Krakowie, ul. Balicka 122, 30-149 Kraków; e-mail: monika.cioch@ urk.edu.pl

Table 1. Main toxic factors occurring in beer

Source: [13]

Źródło: [13]

FUNGI AS MYCOTOXIN PRODUCERS

The word "mycotoxin" descends from a combination of Greek word *mykes* (fungus) and a Latin term for toxin, *toxicum*. This term refers to one of the groups of natural secondary metabolites, characterized by low molecular weight (usually below 1 kDa) produced by some mold fungi [27]. Vertebral organisms can be exposed to them by consumption of contaminated food, by inhalation or by skin contact [10]. They exhibit a wide range of activities, such as carcinogenic, mutagenic, teratogenic, cytotoxic, neurotoxic, nephrotoxic, neurotoxic, immunosuppressive and estrogenic [27]. Their toxicity is dependent on the type of the toxin itself, dose, time of exposition and additional synergistic reactions between other mycotoxins, which may be present in the product [27]. Exposure to high concentrations can have fatal effects, which event that took place in 1944 is an example of. In that year, in the Orenburg region of Russia cereal grains were contaminated with toxigenic species of *Fusarium* (producing T-2 toxin and Trichothecenes A). 10% of the region's population were affected, among whom mortality rate was nearly 60%. Leukemia, bleeding from nose, throat, and gums, necrotic angina, sepsis, rash and fever were observed in the victims. Nowadays however, such contamination in the brewing industry is improbable. The much more worrisome issue is chronic exposure to low doses of mycotoxin, which effects are still vague. Long term intake of aflatoxins, one of the groups of mycotoxins may serve as an example. It is suspected that such an exposure can cause hepatocellular carcinomas, reduction of male fertility, lowering the body's immunity and pulmonary fibrosis [10, 26]. There have been reports stating their contribution to the onset of Ray and Kwashiorkor syndromes in children.

Intensive research into understanding mycotoxins were initiated by accident that took place in 1960, when 100,000 farm birds have died as a result of contamination of the feed by *Aspergillus flavus* [15]. It is currently rated that nearly 350 species of mold have the capacity to produce mycotoxins, whose number is estimated at over 400 [15]. They are formed as a result of significant precursor accumulation required in primary metabolism performance such as amino acids, acetates and pyruvates. The main reason for their synthesis is believed to be cells tendency to reduce the amount of precursors present within them [15].

Mycotoxins are characterized by a high resistance to the effects of environmental factors, thus they can survive the whole production process, during which conditions are not sufficient to render them harmless – so they find the way to the finished product. The most common and also the most dangerous mycotoxins that can be present in beer are:

- Aflatoxins, in particular Aflatoxin B1 (B1) which is believed to have the highest carcinogenic activity among natural toxins. AFB1 is produced by members of *Aspergillus* section *flavi*, especially *Aspergillus flavus*, which are commonly found on aerial parts of plants. These mycotoxins are very stable, as they retain their properties for a long time, even when subjected to processes such as cooking, baking, roasting or extrusion, although alkaline environment tends to reduce their toxicity. Aflatoxins have been recognized by the International Agency for Research on Cancer (IARC) as carcinogenic factors belonging to group 1. AFB1 has hepatotoxic, mutagenic, carcinogenic and immunotoxic activities. They don't have a fixed Tolerable Daily Intake (TDI) value [4,10,15].
- Trichothecenes, including Deoxynivalenol (DON also known as Vomitoxin), Nivalenol (NIV), T-2 toxin (T-2) and HT-2. *Fusarium sporotrichioides*, *F. langsethiae*, *F. poae* are the most important producers of T-2 and HT-2 toxins. The jointly Tolerable Daily Intake for both T-2 and HT-2 toxin is $1 \mu g / 1$ kg body weight per day. They act as immunotoxic and hepatotoxic agents [4]. DON is mainly synthesized by *F. graminearum*, *F. Culmorum* and *F. cerealis*. It has a TDI of 1 µg / 1 kg bw. per day. It is held responsible for diarrhea, vomiting and immunotoxic activity [4, 5].
- Ochratoxin A (OTA), produced mainly by *Aspergillus* section *Circumadati*, *Aspergillus* section *Nigiri*, *Penicillium verrucosum* and *Penicillium nordicum*. Reduction of its content in a raw material takes place when material is treated with temperatures of 250°C for several minutes [7, 10]. OTA is classified by IARC as a possible carcinogen to humans, with it's TDI set to $1 \mu g / 1$ kg bw. per day.
- Fumonisins (FMB1, FMB2, FMB3), mainly produced by molds of the *Fusarium* genus, especially those originating from section *Liseola*. *Fusarium verticillioides* and *F. proliferatum* are considered the most important producers of these mycotoxins. In order to reduce their amount, raw materials should be treated with temperatures of at least 150°C. The Provisional Maximum Tolerable Daily Intake (PMTDI) for the most common toxin from this group, FMB1 is $2 \mu g / 1$ kg bw. per day. It is considered as carcinogen and nephrotoxic agent [4].

• Zearalenone (ZEN), synthetized by molds of the *Fusarium* genus, mainly *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti*, *F. verticillioides* and *F. incarnatum*. Similarly to FMB1, thermal decomposition of ZEN takes place in an environment with temperatures above 150°C [10]. It hasn't been classified by IARC, but TDI for this mycotoxin is 0,25 µg / bw. per day. ZEN shows estrogenic and reprotoxic activities [4].

MYCOTOXIN ORIGIN IN BEER

Traditionally beer is made from 4 main ingredients: water, malt, yeasts and hops. Contrary to the popular belief about strong antiseptic properties of hops, this raw material might also be a source of mycotoxins [21]. However, due to the amount of hops used in the brewing process, and very low quantities of mycotoxin found in the material itself, its influence may be neglected. The main source of those compounds in beer is clearly the brewer's malt. According to FAO, nearly 25% of all cereals grown in the world can be contaminated with mycotoxins, although newer research indicates that the number of infected cereals can actually reach up to 72% [3]. Cereal contamination can take place at various stages of the production process. Incorrect drying, storage and packaging conditions, as well as improper agricultural practices may promote mold fungi growth. Other factors, such as using fungicides in insufficient doses can promote the growth of strains which are able to produce mycotoxins at higher rate [24]. Even if grain has not been contaminated with mycotoxin, due to the ubiquity of spores in the environment, it probably contains fungal spores. Fungal molds found on cereal grains can be divided into two major categories: those infecting plants before the harvest and fungi developing during storage of the grain. The most commonly found organisms of the first group are: *Alternaria*, *Aureobasidium*, *Cladosporium*, *Epicoccum*, *Fusarium* and *Helminthosporium*. Organisms in this group are parasites or saprophytes, that contribute to significant crop losses. The growth of these specific microorganisms is highly dependent on climatic conditions, varieties of cultivated cereals and agricultural practices. Fungi belonging to the second group are absent or identified only in small quantities in fresh raw material, and their quantity increases over storage time. The most common microorganisms in the second group are those of *Aspergillus* genus, and to a lesser degree, *Penicillium*. Storing grain with an increased moisture content may lead to development of the molds from *Absidia*, *Rhizopus* and *Mucor* genus [6]. These microorganisms possess a threat to all cultivated cereals, but because the most commonly used cereal in the brewing industry is barley, the remainder of the paragraph will focus on this cereal. The main threat to brewer's barley quality is the growth of filamentous fungi of *Fusarium* genus.

In order to obtain from the grain the material required for the production of beer, grains are subjected to the process known as malting. The purpose of this treatment is to change the physical structure and chemical composition of the grain through stimulation of the natural process of germination, and terminating it at the right moment. First problem arising at this stage is reduced germination capacity of contaminated grains. Actions taken during the malting process, create favorable conditions for the growth and production of mycotoxins by

mold fungi. Wide availability of nutrients, water immersion of grains combined with aeration, low temperatures during steeping and germination and high relative humidity $(\sim 90\%)$ stimulate growth of the fungal spores that might be present in the raw material. Microbial growth during this process highly depends on initial contamination of the grain, possible interactions between different microorganisms present in the environment, nutrient availability and the applied conditions of the malting process, such as temperature, humidity and rate of aeration. Additional contamination might come from endogenous microflora of a malthouse [22]. Fungal growth and as a result mycotoxin synthesis takes place during the whole malting process, until freshly obtained malt is dried to a water content of 4–5% [10,24]. Proper conduct of the malting process can lead to significant reduction of water-soluble toxins such as DON and ZEN. According to Piacentini et. all [17], provided there is no secondary growth of mycelium, malting can reduce the ZEN and DON levels by 69 and 71% respectively, compared to raw materials. Content of other toxins, such as T-2 and HT-2 is also reduced during the process [10]. Grains tested immediately after kilning are characterized by the lowest amounts of those compounds, which rises on the next steps of the malting process. In case of severe grains contamination, the final concentration of these toxins in malt can be even twice as high as in the raw material [10, 17]. It is noteworthy that the reduction of some mycotoxin content might be ostensible due to so-called modified mycotoxins. These toxins are conjugated to more polar compounds, such as sugars, and they are thought to be less toxic to the living organisms. As for now, the detailed information regarding their toxicity is still unknown, but it has been documented that the bonds with polar compounds and the toxin itself can be broken down inside digestive tract by the activity of lactic acid bacteria, which naturally inhabits mammal gastrointestinal tract, freeing toxins in the process [3]. Research suggests up to 50% of DON present in barley grains can be biotransformed to DON-3-Glc conjugate, thanks to activity of enzymes activated during malting [17]. Similar mechanism also occurs in cereal grains before harvest, probably as a specific way of "detoxifying" plants from fungal metabolites. It is considered that other mycotoxins produced by *Fusarium*, such as ZEN, FMB1, T-2, HT-2 and NIV can also undergo bonding with polar compounds [3]. As modified mycotoxins are more water soluble, there is a risk they can pass in larger quantities into the wort during the mashing process than original compounds.

According to research conducted by Gonzalez Pereyra et. all [6], the most common microorganisms found in malt are mold fungi from *Fusarium*, *Geotrichum* and *Aspergillus* genus. *Penicillium*, *Cladosporium* and *Alternaria* were also found, but less frequently. The most common representatives of *Fusarium* genus were *F. verticilloidies* and *F. proliferatum*. All of the malts tested in this research contained 104–145 µg/ kg FMB1. AFB1 was detected in 18% of samples, at 19–44, 52 µg/kg. Malt contamination with AFB1 is relatively rare in European cultivations, since mold fungi producing them prefer a warmer climate [22]. As mentioned earlier, nearly all of the cereal is contaminated by mycotoxins to some degree, so the exact amount of compounds which passes from malt to a finished product is an important issue. Results obtained by Piacentini et. all [17] shows that on average, about 91% of DON contained in malt grains goes to the wort, and 89,9%

into the finished products. For ZEN first value is 6,3%, and in the finished product it's content is below detection level.

MYCOTOXIN INFLUENCE ON BEER SENSORY CHARACTERISTICS AND PRODUCTION PROCESS

The fungal metabolites load of the malt influences the fermentation process of the wort and the sensory characteristics of the finished beer. The main problem associated with usage of contaminated grains is a phenomenon known as gushing. It manifests itself in a violent and intensive foam formation after opening a beverage, resulting in a significant loss of the product. Contamination of the malt with a peptide metabolites of fungi, known as hydrophobins is indicated as the main cause of this defect. Other possible sources of this flaw are: bottle contamination, cleaning agent residues, excessive carbonation and occurence of metal ions or oxalates in the finished product [24]. Hydrophobins probably stabilize carbon dioxide bubbles in the drink by forming a protective layer around them, preventing them from collapsing, which leads to the creation of excessive amounts of foam [20]. The species responsible for this phenomenon are primarily those from *Fusarium* genus, however research suggests this defect may also be caused through development of *Aspergillus*, *Penicillium*, *Nigrospora* and *Stemphylium* [20,24]. It is believed that unlike mycotoxins, all mold fungi species can synthetize hydrophobins, so the content of the former can't be a direct determinant of the possibility of this defect appearing. It is also worth noting that fungi can also produce compounds counteracting gushing, such as lipids, therefore the resultant effect of such contamination is difficult to determine [20].

Fungal metabolites may affect wort quality parameters (such as Free Amino Acid content – FAN, pH, color), course of fermentation and parameters of finished product (FAN, color, flavor and aroma) [22]. Contaminated malt can be a source of thermostable proteases that, acting with endogenous malt enzymes will break down bonds inside malt proteins to a higher degree than endogenous enzymes themselves. This may cause changes to beer color, texture, aroma, flavour and foaming. Increased protease activity of the mash can accelerate fermentation rate thanks to higher FAN content in wort. However, the presence of T-2, DON and ZEN is known to reduce the rate of fermentation [22, 24]. The growth of fungi can also increase the amount of β-glucanases and pentosanases in malt. Additional activities from these enzymes could increase the efficiency and speed of wort filtration [22].

It has long been known that the growth of *Fusarium* is related to formation of undesirable aromas in beer [22]. In the research conducted by Oliveira et. all [12] beers brewed with highly contaminated malts contained higher content of volatile compounds, respectively: 10% more higher alcohols, 10% esters, 40% fatty acids, 75% ketones, 100% dimethyl sulfide and 1300% acetaldehyde, compared to the control sample. The authors suggest that an increase of higher alcohols, esters and ketones content is associated with a higher concentration of FAN in the wort obtained from contaminated malt. A significant increase in acetaldehyde concentration indicates a deficiency of active yeast cells in the final stages of fermentation. It indicates a decrease in yeast viability and premature flocculation. Additionally, quality parameters of obtained beer indicate it might be more susceptible to aging processes [12].

DECREASING MYCOTOXIN CONTENT USING TECHNOLOGICAL PROCESSES

The most beneficial solution in terms of beer quality and consumer health would be to completely stop using grain suspected of developing mold. However, in some years avoiding microbial infection is nearly impossible, due to the atmospheric conditions. Crop cultivations in some countries are especially prone to infections, as warm and humid climate promote them. Even healthy grains can be contaminated as a result of improper storage conditions or mistakes during malting. European Commission regulations sets the legal limits for maximum admissible content of mycotoxins in grains, namely: 2 µg/kg grain for AFB and 4 µg/kg for the sum of all aflatoxins, 100 µg/kg for ZEN, 1250 µg/kg for DON and 2000 µg/kg for sum of FMB1 and FMB2 [14]. But these values may be exceeded during the malting process. Malthouses should make every effort in order to ensure proper quality and health safety of their products. Proper malting conditions, such as steeping with restricted aeration, water change during aeration breaks, strict control of temperature during germination as well as general care for the equipment cleanliness can significantly reduce mold growth [22]. Since the temperatures used in the brewing process are not high enough to destroy toxins, another solution is needed. The most beneficial solution counteracting this problem in terms of economy and consumer health is decontamination. It's purpose is to reduce the amount of fungal spores present in the raw material along with reducing their growth during the process. Decontamination methods include:

- Ozonation using 5 minute ozone treatment under certain conditions allows to deactivate 96% of mold spores, without decrease in grain's germination capacity (fungal cells are less resistant to ozone than seed embryos) and without leaving any residue [14];
- Washing the grains with hot water before malting $$ treatment with water at 45°C can reduce the amount of water soluble toxins such as DON in finished malt by 79– 93% [14],
- Steeping using chlorinated water, water enriched with addition of hydrogen peroxide or alkaline waters – those methods allow to reduce the spore load of raw material, although they are cost-ineffective. Furthermore, using too high concentrations of this chemicals may decrease germination capacity or cause adverse sensory characteristics of malt [22];
- Addition of selected strains of lactic acid bacteria (LAB) – mainly those from *Lactobacillus* or *Pediococcus* genus have the ability to inhibits growth of *Fusarium* up to 23%, which may lead to 83% decrease in DON levels in brewer's malt. Production of lactic acid by these organisms, resulting in lowering of pH may increase enzymatic activity during malting and mashing [14];
- Addition of selected *Geotrichum candidum* cultures – growth of this microorganism confines the growth

of undesirable molds from *Fusarium*, *Penicillium* and *Aspergillus* genus, inhibits their ability to produce metabolites and stimulates growth of lactic acid bacteria. LAB further inhibits fungi growth by lowering the pH of the environment. Brewer's malt obtained in this way has superior quality parameters such as more effective filtration, inhibition of polysaccharide producing microflora and reduced fatty acids content [22];

Radiation method – electron beam irradiation can reduce brewer's malt DON content by 60-100% with minimal impact on quality parameters of the product, leaving no residue [14]. This method relies heavily on using the right amount of radiation energy. Too low energy might not be sufficient to dispose of all of the fungal spores, and remaining microorganisms may have increased ability to produce secondary metabolites, as it is seen with *Aspergillus flavus* and *A. parasiticus*. On the other hand, too high energy significantly reduces germination capacity and changes the malt quality parameters [14,22].

Reduction of mycotoxin levels also takes place during the beer production stage. This can occur by removing them from the product or by transformation into less toxic forms. Removal of mycotoxins from the product can occur by binding them in sludge, which is separated from the product. Although not tested, it is believed there is a possibility of binding them with clarifying agents [14]. It is known that removal of OTA, ZEN and AFB1 occurs naturally by binding these compounds to β-glucans present in the cellular wall of the *Saccharomyces cerevisiae*. This phenomenon might allow to reduce wort ZEN levels up to 75,1 % [17]. Dead yeast cells have a higher binding capacity than live cells [14]. In current brewing technology, yeast sludge as well as live cells suspended in beer, are filtered out before bottling, which combined with mentioned phenomenon allows to obtain a product partly deprived of mycotoxins. Some of the microorganisms are known for their ability of their biotransformation into less harmful compounds. Certain *S. cerevisiae* strains are able to breakdown ZEN, FMB1 and FMB2, although it is a relatively slow process. Non-*Saccharomyces* yeasts, such as *Candida tropicalis*, *Torulaspora delbrueckii* and *Zygosaccharomyces rouxii* can transform ZEN into less dangerous β-ZEN [22].

MYCOTOXIN OCCURRENCE IN BEERS ON EUROPEAN MARKET

Currently, European Union legislation regulates the occurrence of 13 mycotoxins in food products (Commission Regulation 1881/ 2006 and 2013/165/EU Recommendation). Beer belongs to the category of cereal-based products, for which the limits are: 2 µg/kg for AF1, 750 µg/kg for DON, 75 µg/kg for ZEN, 400 µg/kg for the sum of FMB1 and FMB2, 5 µg/kg for OTA [14]. The data in Table 2 shows that the most common mycotoxin in beers on European market is DON. Depending on the source, its presence was found in 40,6-100% of tested beers. Kuzdraliński et al. [9] reports an average DON content range found in beers of 6,0-70,2 µg/l. Additionally DON was found in all of 57 samples originating from Poland. In studies conducted by Bryła et. al. [2] on the domestic market, the average DON concentration was 9.0 ± 12.7 μ g/l. The authors also investigated occurence of modified form of DON, DON-3-Glc. It's concentration was on average 9,2±7,5 µg/l. In a five-year study conducted by Olšovská et al. [13] ZEN was detected in only one sample from a pool of 157. When ZEN contamination occurs, its average content is in the range of $0,259-0,546 \mu g/l$ [9]. T-2 and HT-2 are relatively rarely found in beer. In samples they were present, their total level was in the range of 0,3-0,85 µg/l [13]. Despite nearly 70% contamination of beers with OTA, according to Bertuzzi et al. [1], the average content of this toxin is relatively low $(0,019\pm0,029 \,\mu$ g/l). Similar results were found by Olšovská et al. [13]. As molds from *Fusarium* genus are the most common fungal microorganism found on cereal grains, significant beer contamination with FMB1 and FMB2 is not surprising. Average amounts of these mycotoxins found by Bertuzzi et. all was $5,8\pm7,4$ µg/l for FMB1 and $0,6\pm1,0$ µg/l for FMB2. The average NIV content in beers containing them is $2,4\pm1,9$ µg/l, as reported by Bryła et al. [2].

Studies also show significant differences in the mycotoxin levels depending on beer style, raw materials used for its production, fermentation method and its alcohol content. Results obtained by Peters et al. [16] indicate higher DON and DON-3-GLC amounts in beers belonging to Imperial Bock and Eisbock styles. Contaminations were also found more frequently in beverages from those styles. On the contrary,

Table 2. Prevalence of mycotoxin contamination in beer. The results refer to beers available on the European market. **Individual columns present the contribution of samples in which mycotoxins were found**

Tabela 2. Częstość występowania skażenia mykotoksynami w piwie. Wyniki dotyczą piw dostępnych na rynku europej-			
skim. Poszczególne kolumny przedstawiają udział prób w których stwierdzono obecność mykotoksyn			

Source: [1, 2, 9, 11, 13, 19] **Źródło:** [1, 2, 9, 11, 13, 19] lowest mycotoxin levels were found in Saison, Pale lager and other styles with low alcohol content. As the alcohol concentration in beer increases, an exponential increase in DON concentration is observed [8]. The probable explanation for this phenomenon is the characteristics of a strong beer production process – along with higher quantities of malt required to reach assumed alcohol levels, larger amounts of mycotoxins are supplied into the wort [8,18]. Many researchers observed higher concentrations of DON and HT-2 in wheat beers. This is probably due to the more frequent occurrence of these mycotoxin producers on wheat grains than on barley, hence all styles based on wheat malt are more exposed to increased HT-2 and DON levels [1]. A slightly higher DON contamination is reported in top fermented and dark beers, regardless of the alcohol content [9].

Despite the widespread occurrence of fungal metabolites in beverages, their content in most cases does not exceed the prescribed standards. According to Rodríguez-Carrasco et al. [19], the average DON and HT-2 content is 24,5-47,7 µg/l and 24,2-38,2 µg/l respectively. Considering average *per capita* consumption in Europe of 70,1 l/year, and assuming a body weight of 70 kg (standard average body weight established by European Food Safety Authority), the statistical consumer drinks 0,192 l of beer per day, thus providing this way 5% PMTDI of DON and 7-12% TDI of HT-2. In countries where consumption is higher, such as the Czech Republic, these values are 9 and 14-24% TDI respectively. The situation of people who consume excessive amounts of alcohol can raise concerns. Assuming consumption of 1 l of beer, the consumer can deliver in this way 25% PMTDI of DON and 37-64% TDI HT-2 [1]. It should also be noted that there are particularly contaminated beers on the market. Peters et al. [16] found beers samples in which the DON content ranged from 225 μ g/l to 1031 μ g/l. In this case, consumption of only 0,33 l such beer can significantly exceed TDI.

REFERENCES

- **[1] BERTUZZI T., S. RASTELLI, A. MULAZZI, G. DONADINI, A. PIETRI. 2011.** "Mycotoxin occurrence in beer produced in several European countries". Food Control 22(12): 2059–2064.
- **[2] BRYŁA M., E. KSIENIEWICZ-WOŹNIAK, A. WAŚKIEWICZ, K. SZYMCZYK, R. JĘDRZEJ-CZAK. 2018.** "Co-occurrence of nivalenol, deoxynivalenol and deoxynivalenol-3-glucoside in beer Samales". Food Control 92: 319–324.
- **[3] DALL'ASTA C.F., F. BERTHILLER. 2015.** "Masked Mycotoxins in Food: Formation, Occurrence and Toxicological Revelance". Cambridge, Royal Society of Chemistry.
- **[4] DEGEN G.H., F. PARTOSCH, K. MUÑOZ, U. GUNDERT-REMY. 2017.** "Daily uptake of mycotoxins – TDI might not be protective for nursed infants". Toxicology Letters 277: 69–75.
- **[5] EFSA PANEL ON CONTAMINANTS IN THE FOOD CHAIN (CONTAM). 2011***.* "Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed". EFSA Journal 9(12): 2481.

CONCLUSION

Despite the frequent occurrence of mycotoxins in beers on the European market, with some exceptions, the level of contamination of these products is relatively low. Particularly noteworthy are strong beers, especially Imperial Stout, which are characterized by a much higher occurrence of fungal mycotoxins than beers with a standard alcohol content. Beers of these styles are usually not produced by large concerns, which is why, with the development of the craft beer industry, more and more beverages with a significant alcohol content appear on the market. Their producers and malt suppliers should pay particular attention to the mycotoxin occurrence in the finished product and, if required, take measures to reduce their content. Good hygiene and production practices, from raw material to finished product, are necessary to maintain high product quality.

PODSUMOWANIE

Mimo częstego występowania mykotoksyn w piwach na rynku europejskim, za pewnymi wyjątkami, poziom skażenia tych produktów jest stosunkowo niski. Szczególną uwagę zwracają piwa mocne, a w szczególności Imperial Stout, charakteryzujące się znacznie wyższą zawartością toksyn grzybowych, niż piwa o standardowej zawartości alkoholu. Piwa w tym stylu nie są zazwyczaj produkowane przez duże koncerny, dlatego też wraz z rozwojem branży piw kraftowych, na rynku pojawiać się może coraz więcej napojów o znacznej zawartości alkoholu. Ich producenci oraz dostawcy słodu powinni zwrócić szczególną uwagę na zawartość mykotoksyn w gotowym produkcie i jeżeli to wymagane, podjąć działania mające na celu obniżenie ich zawartości. Dobre praktyki higieniczne i produkcyjne, od surowca, aż do produktu gotowego, są niezbędne do utrzymania wysokiej jakości wyrobu.

REFERENCES

- **[1] BERTUZZI T., S. RASTELLI, A. MULAZZI, G. DONADINI, A. PIETRI. 2011.** "Mycotoxin occurrence in beer produced in several European countries". Food Control 22(12): 2059–2064.
- **[2] BRYLA M., E. KSIENIEWICZ-WOZNIAK, A. WASKIEWICZ, K. SZYMCZYK, R. JEDRZEJ-CZAK. 2018**. "Co-occurrence of nivalenol, deoxynivalenol and deoxynivalenol-3-glucoside in beer Samales". Food Control 92: 319–324.
- **[3] DALL'ASTA C.F., F. BERTHILLER. 2015.** "Masked Mycotoxins in Food: Formation, Occurrence and Toxicological Revelance". Cambridge, Royal Society of Chemistry.
- **[4] DEGEN G.H., F. PARTOSCH, K. MUNOZ, U. GUNDERT-REMY. 2017.** "Daily uptake of mycotoxins – TDI might not be protective for nursed infants". Toxicology Letters 277: 69–75.
- **[5] EFSA PANEL ON CONTAMINANTS IN THE FOOD CHAIN (CONTAM). 2011.** "Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed". EFSA Journal 9(12): 2481.
- **[6] GONZALEZ PEREYRA M.L, C.A.R. ROSA, A.M. DALCERO, L.R. CAVAGLIERI. 2011.** "Mycobiota and mycotoxins in malted barley and brewer's spent grain from Argentinean breweries". Letters in Applied Microbiology 53: 649–655.
- **[7] INOUE T., Y. NAGATOMI, A. UYAMA, N. MO-CHIZUKI. 2013.** "Fate of Mycotoxins during Beer Brewing and Fermentation". Bioscience, Biotechnology and Biochemistry 77(7): 1410–1415.
- **[8] KOSTELANSKA M., J. HAJSLOVA, M. ZA-CHARIASOVA, A. MALACHOVA, K. KALAC-HOVA, J. POUSTKA, J. FIALA, P.M. SCOTT, F. BERTHILLER, R. KRSKA. 2009.** "Occurrence of Deoxynivalenol and Its Major Conjugate, Deoxynivalenol-3-Glucoside, in Beer and Some Brewing Intermediates". Journal of Agricultural and Food Chemistry 57(8): 3187–3194.
- **[9] KUZDRALIŃSKI A., E. SOLARSKA, M. MU-SZYŃSKA.** 2013. "Deoxynivalenol and zearalenone occurence in beers analysed by an enzyme–linked immunosorbent assay method". Food Control 29(1): 22–24.
- **[10] MARIN S., A.J. RAMOS, G. CANO–SANCHO, V. SANCHIS. 2013.** "Mycotoxins: Occurrence, toxicology, and exposure assessment". Food and Chemical Toxicology 60: 218–237.
- **[11] MASTANJEVIĆ K., J. LUKINAC, M. JUKIĆ, B. ŠARKANJ, V. KRSTANOVIĆ, K. MASTANJE-VIĆ. 2019.** "Multi-(myco)toxins in Malting and Brewing By-Products". Toxins 11(1): 30.
- **[12] OLIVEIRA P., A. M,AUCH, J. FRITZ, E.K.** ARENDT. 2012. "Impact of *Fusarium*Culmorum-Infected Barley Malt Grains on Brewing and Beer Quality". Journal of the American Society of Brewing Chemists 70(3): 186–194.
- **[13] OLŠOVSKÁJ.,V. JANDOVSKÁ, S.BĔLÁKOVÁ, P. KUBIZNIAKOVÁ, T. VRZAL, K. ŠTĔRBA.** 2019. "Monitoring of potential contaminants in beer from Czech Republic". KvasnyPrumysl 65(3): 84–96.
- **[14] PASCARI X., A.J. RAMOS, S. MARÍN, V. SAN-CHÍS. 2018.** "Mycotoxins and beer. Impact of beer. production process on mycotoxin contamination. A review". Food Research International 103: 121–129.
- **[15] PERDONCINI M.R.F.G., M.J. SEREIA, F.H.P. SCOPEL, M. FORMIGONI, E.S. RIGOBELLO, S.C. BENETI, F.A.R. CARDOSO, L.B.MARCHI, C. GOMES DE SILVA JUNIOR, P.G.M. FER-NANDES, T.H. SMIELEVSKI DE SOUZA, P. WIELEWSKI, E. GOMES DE LIMA, A. GRE-GÓRIO, M.R.T. ZORZENON, J.C. CASTRO, V. DE CÁSSIA MENDES BEL DEL, M. SOARES DOS SANTOS POZZA, L.L.M. MARQUES.** 2018. "Growth of Fungal Cells and the Production of Mycotoxins".
- **[6] GONZALEZ PEREYRA M.L, C.A.R. ROSA, A.M. DALCERO, L.R. CAVAGLIERI. 2011.** "Mycobiota and mycotoxins in malted barley and brewer's spent grain from Argentinean breweries". Letters in Applied Microbiology 53: 649–655.
- **[7] INOUE T., Y. NAGATOMI, A. UYAMA, N. MO-CHIZUKI. 2013.** "Fate of Mycotoxins during Beer Brewing and Fermentation". Bioscience, Biotechnology and Biochemistry 77(7): 1410–1415.
- **[8] KOSTELANSKA M., J. HAJSLOVA, M. ZA-CHARIASOVA, A. MALACHOVA, K. KALAC-HOVA, J. POUSTKA, J. FIALA, P.M. SCOTT, F. BERTHILLER, R. KRSKA. 2009.** "Occurrence of Deoxynivalenol and Its Major Conjugate, Deoxynivalenol-3-Glucoside, in Beer and Some Brewing Intermediates". Journal of Agricultural and Food Chemistry 57(8): 3187–3194.
- **[9] KUZDRALINSKI A., E. SOLARSKA, M. MU-SZYNSKA.** 2013. "Deoxynivalenol and zearalenone occurence in beers analysed by an enzyme–linked immunosorbent assay method". Food Control 29(1): 22–24.
- **[10] MARIN S., A.J. RAMOS, G. CANO–SANCHO, V. SANCHIS. 2013.** "Mycotoxins: Occurrence, toxicology, and exposure assessment". Food and Chemical Toxicology 60: 218–237.
- **[11] MASTANJEVIC K., J. LUKINAC, M. JUKIC, B. SARKANJ, V. KRSTANOVIC, K. MASTANJE-**VIC. 2019. "Multi-(myco)toxins in Malting and Brewing By-Products". Toxins 11(1): 30.
- **[12] OLIVEIRA P., A. M,AUCH, J. FRITZ, E.K.** ARENDT. 2012. "Impact of FusariumCulmorum-Infected Barley Malt Grains on Brewing and Beer Quality". Journal of the American Society of Brewing Chemists 70(3): 186–194.
- **[13] OLSOVSKA J., V. JANDOVSKA, S. BELAKOVA, P. KUBIZNIAKOVA, T. VRZAL, K. STERBA.** 2019. "Monitoring of potential contaminants in beer from Czech Republic". KvasnyPrumysl 65(3): 84–96.
- **[14] PASCARI X., A.J. RAMOS, S. MARIN, V. SAN-CHIS. 2018.** "Mycotoxins and beer. Impact of beer. production process on mycotoxin contamination. A review". Food Research International 103: 121–129.
- **[15] PERDONCINI M.R.F.G., M.J. SEREIA, F.H.P. SCOPEL, M. FORMIGONI, E.S. RIGOBELLO, S.C. BENETI, F.A.R. CARDOSO, L.B.MARCHI, C. GOMES DE SILVA JUNIOR, P.G.M. FER-NANDES, T.H. SMIELEVSKI DE SOUZA, P. WIELEWSKI, E. GOMES DE LIMA, A. GRE-GORIO, M.R.T. ZORZENON, J.C. CASTRO, V. DE CASSIA MENDES BEL DEL, M. SOARES DOS SANTOS POZZA, L.L.M. MARQUES.** 2018. "Growth of Fungal Cells and the Production of Mycotoxins".
- **[16] PETERS J., R. VAN DAM, R. VAN DOORN, D. KATARERE, F. BERTHILLER, W. HAASNOOT, M.W.F. NIELEN. 2017.** "Mycotoxin profiling of 1000 beer samples with a special focus on craft beer". Plos One 12(10).
- **[17] PIACENTINI K.C., S. BĔLÁKOVÁ, K. BENEŠO-VÁ, M. PERNICA, G.D. SAVI, L.O. ROCHA, I. HARTMAN, J. ČÁSLAVSKÝ, B. CORRȆA, S. BĚLÁKOVÁ.** 2019. "*Fusarium* Mycotoxins Stability during the Malting and Brewing Processes". Toxins 11(5): 257.
- **[18] RAI M., A. VARMA (Eds.). 2010.** Mycotoxins in Food, Feed and Bioweapons. Berlin, Springer.
- **[19] RODRÍGUEZ-CARRASCO Y., M. FATTORE, S. ALBRIZIO, H. BERRADA, J. MAÑES. 2015.** "Occurrence of *Fusarium* mycotoxins and their dietary intake through beer consumption by the European population". Food Chemistry 178: 149–155.
- **[20] SARLIN T., T. NAKARI-SETÄLÄ, M. LINDER, M. PENTTILÄ, A. HAIKARA. 2005.** "Fungal Hydrophobins as Predictors of the Gushing Activity of Malt". Journal of the Institute of Brewing 111(2): 105–111.
- **[21] SCHMIDT R., WOLNZACH, P. ANDEREGG, ZÜRICH, M. BIENDL. 2004.** "Environmental contaminants in hops". Brauwelt International 5:302– 305.
- **[22] TANGNI E.K., Y. LARONDELLE. 2002.** , Malts, moulds and mycotoxins". Bacteria, Yeasts and Moulds in Malting and Brewing: Proceedings of the Xth Symposium "Chair J. de Clerck". Lauven (Belgium).
- **[23] THE BREWERS OF EUROPE. 2019.** "European beer trends. Statistic Report". Belgium, The Brewers of Europe.
- [24] **WOLF-HALL C.E. 2007.** "Mold and mycotoxin problems encountered during malting and brewing". International Journal of Food Microbiology 119(1–2): 89–94.
- **[25] WORLD HEALTH ORGANISATION. 2018***.* "Global status report on alcohol and health 2018". Switzerland, World Health Organization.
- **[26] ZAIN M.E. 2011.** Impact of mycotoxins on humans and animals". Journal of Saudi Chemical Society 15(2): 129–144.
- **[27] ZHIQIANG A. (Ed.). 2004**. "Handbook of Industrial Mycology". New York: CRC Press.
- **[16] PETERS J., R. VAN DAM, R. VAN DOORN, D. KATARERE, F. BERTHILLER, W. HAASNOOT, M.W.F. NIELEN. 2017.** "Mycotoxin profiling of 1000 beer samples with a special focus on craft beer". Plos One 12(10).
- **[17] PIACENTINI K.C., S. BELAKOVA, K. BENE-SOVA, M. PERNICA, G.D. SAVI, L.O. ROCHA, I. HARTMAN, J. CASLAVSKY, B. CORREA, S. BELAKOVA. 2019.** "Fusarium Mycotoxins Stability during the Malting and Brewing Processes". Toxins 11(5): 257.
- **[18] RAI M., A. VARMA (Eds.). 2010.** Mycotoxins in Food, Feed and Bioweapons. Berlin, Springer.
- **[19] RODRIGUEZ-CARRASCO Y., M. FATTORE, S. ALBRIZIO, H. BERRADA, J. MANES. 2015.** "Occurrence of Fusarium mycotoxins and their dietary intake through beer consumption by the European population". Food Chemistry 178: 149–155.
- **[20] SARLIN T., T. NAKARI-SETALA, M. LINDER, M. PENTTILA, A. HAIKARA. 2005.** "Fungal Hydrophobins as Predictors of the Gushing Activity of Malt". Journal of the Institute of Brewing 111(2): 105–111.
- **[21] SCHMIDT R., WOLNZACH, P. ANDEREGG, ZURICH, M. BIENDL. 2004.** "Environmental contaminants in hops". Brauwelt International 5:302– 305.
- **[22] TANGNI E.K., Y. LARONDELLE. 2002.** "Malts, moulds and mycotoxins". Bacteria, Yeasts and Moulds in Malting and Brewing: Proceedings of the Xth Symposium "Chair J. de Clerck". Lauven (Belgium).
- **[23] THE BREWERS OF EUROPE. 2019.** "European beer trends. Statistic Report". Belgium, The Brewers of Europe.
- [24] **WOLF-HALL C.E. 2007.** "Mold and mycotoxin problems encountered during malting and brewing". International Journal of Food Microbiology 119(1–2): 89–94.
- **[25] WORLD HEALTH ORGANISATION. 2018.** "Global status report on alcohol and health 2018". Switzerland, World Health Organization.
- **[26] ZAIN M.E. 2011**. Impact of mycotoxins on humans and animals". Journal of Saudi Chemical Society 15(2): 129–144.
- **[27] ZHIQIANG A. (Ed.). 2004.** "Handbook of Industrial Mycology". New York: CRC Press.