Review article
DOI: /10.5513/JCEA01/24.1.3704
Journal of Central European Agriculture, 2023, 24(1), p.137-150

Occurrence of mycotoxins in food and beverages

Pojavnost mikotoksina u hrani i piću

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Received: August 1, 2022; accepted: November 20, 2022

ABSTRACT

This paper describes the mycotoxins produced by fungi from the genera *Fusarium*, *Penicillium*, *Aspergillus*, *Claviceps* and other types of molds, their characteristics and importance. Mycotoxins are secondary fungi metabolites that serve as a defense mechanism in stressful conditions. Several hundred mycotoxins have been identified so far, and the most significant in terms of danger to human health and animals are aflatoxins, ochratoxin A, patulin, fumonisin, zearalenone and nivalenol/deoxynivalenol produced by toxigenic fungi from the genera *Penicillium*, *Aspergillus*, *Claviceps*, *Stachybotris* and *Fusarium*. Moisture and temperature are two factors that have a crucial influence on the development of the fungus and the synthesis of toxins. It is estimated that approximately 25% of agricultural crops are infected with fungi. Consequently, foods can also be contaminated with mycotoxins. By treating the stored grain with various chemicals, and in recent years, using environmentally friendly fungicides, the synthesis of mycotoxins is being prevented and the development of mycotoxicogenic fungi is being controlled. The mycotoxicosis can occur directly through the consumption of infected food, inhalation and skin contact, or indirectly through animals that eat contaminated feed. Mycotoxins have a pathological effect primarily on liver. Some mycotoxins also interfere with cellular protein synthesis, causing hypersensitivity and extreme immunodeficiency. Despite mycotoxins being resilient and remaining stable while undergoing chemical and thermal food processing, methods such as applying good hygiene and production practices from field to table can reduce their occurence.

Keywords: aflatoxin, contamination, *Fusarium* sp., good agricultural practice, ochratoxin A, phytopathogenic fungi

SAŽETAK

U ovom radu opisani su mikotoksini koji stvaraju gljivice iz rodova *Fusarium*, *Penicillium*, *Aspergillus*, *Claviceps* i drugih vrsta plijesni; njihove karakteristike i važnost. Mikotoksini su sekundarni metaboliti gljivica koji služe kao obrambeni mehanizam u stresnim uvjetima. Do sada je identificirano više stotina mikotoksina, a najznačajniji s obzirom na opasnost za ljudsko zdravlje i životinje su aflatoksini, ohratoksin A, patulin, fumonizin, zearalenon i nivalenol/deoksinivalenol koje stvaraju toksikogene gljivice iz rodova *Penicillium*, *Aspergillus*, *Claviceps*, *Stachybotris* i *Fusarium*. Vlaga i temperatura dva su faktora koji imaju krucijalni utjecaj na razvoj gljive i sintezu toksina. Procjenjuje se da je oko 25% poljoprivrednih usjeva zaraženo gljivama. Posljedično tome namirnice mogu također biti kontaminirane mikotoksinima. Tretiranjem uskladištenog zrna raznim kemikalijama, a zadnjih godina primjenom ekološki prihvatljivih fungicida, onemogućuje se sinteza mikotoksina i kontrolira se razvoj mikotoksikogenih gljiva. Do pojave mikotoksikoza može doći direktno konzumacijom zaražene hrane, inhalacijom i kontaktom putem kože ili indirektno konzumacijom mesa, mliječnih proizvoda i jaja životinja koje se hrane kontaminiranom krmom. Mikotoksini patološki prije svega djeluju na jetru. Neki mikotoksini također interferiraju sa sintezom staničnih bjelančevina uzrokujući preosjetljivost i ekstremnu imunodeficijenciju. Budući da je karakteristika mikotoksina da su kemijski i termički stabilni i da podnose procese prerade hrane, ipak postoje

DOI: /10.5513/JCEA01/24.1.3704

metode pomoću kojih se njihova pojavnost može smanjiti, pogotovo primjenom dobre higijenske i proizvodne prakse od polja do stola.

Ključne riječi: aflatoksin, kontaminacija, *Fusarium* sp., dobra poljoprivredna praksa, ohratoksin A, fitopatogene gljive

INTRODUCTION

The occurrence of filamentous fungi in food can lead to the release of toxins known as mycotoxins. Mycotoxins are secondary metabolites that can cause various diseases in humans, ranging from allergic reactions to reduced immune system response and cancer (Pitt, 2000).

While primary metabolites of fungi and other microbes are compounds that are essential for their development, secondary metabolites of fungi are being formed in the final periods of their exponential growth. Secondary metabolites are classified based on the starting positions in their production as polyketides, terpenes, substances derived from shikimic acid, and metabolites derived from amino acids. Their structural differences are the result of several reactions such as condensation, oxidation, reduction, alkylation, and halogenation. Fungi produce mycotoxins in stressful conditions, such as inadequate moisture, temperature, poor ventilation and the presence of aggressive factors (water stress that occurs in the field due to prolonged droughts, with a consequent reduction in vigor which often makes plants susceptible to infection and colonization of toxicogenic fungi) (Havranek et al., 2014).

Mycotoxin-producing fungi can grow on a variety of foods such as grains, dried fruits, nuts and spices. Fungal growth can occur before and after the harvest, while food is being in the storage, on/in food in warm and humid conditions. However, one of the characteristics of mycotoxins is that they are chemically stable and can survive food processing processes (WHO, 2018).

Contamination with mycotoxins can occur directly through the consumption of infected food or indirectly through animals that feed on contaminated food, most often milk (WHO, 2018), i.e. through ingestion, inhalation and skin contact. Most cases of mycotoxin infection occur after the consumption of contaminated food (CAST, 2003).

Mycotoxins have no biochemical significance in terms of their influence on the growth of fungi, but they serve as a defense mechanism against insects, microorganisms, nematodes, animals, and humans (Etzel, 2002).

Mycotoxicoses are poisonings caused by toxic metabolites of various fungi, most often molds, and are not transmissible like infectious diseases. Mycotoxins have a pathological effect on almost all organs in the body of animals and humans, for example the liver, kidneys, spleen, mucous membrane of the mouth, digestive and reproductive systems, central and peripheral nervous system. First of all, they have a pathological effect on the liver, because there is where the mycotoxin detoxification process takes place (Havranek et al., 2014). Mycotoxins have four mechanisms of toxic action: acute, chronic, mutagenic and teratogenic (Pitt, 2000).

The most well-known epidemics caused by mycotoxin poisoning are: ergotism, alimentary toxic aleukia, stachybotrytoxicosis, and aflatoxicosis (Pitt, 2000).

Mycotoxicogenic fungi, mycotoxins and their occurrence in food and drink

Several hundred mycotoxins have been identified so far, however, the most significant mycotoxins that pose a threat to human and animal health are aflatoxins, ochratoxin A, patulin, fumonisin, zearalenone and nivalenol/deoxynivalenol (WHO, 2018). Mycotoxins are produced by toxicogenic fungi from the following genera: *Penicillium*, *Aspergillus*, *Claviceps*, *Stachybotris*, *Fusarium* (Havranek et al., 2014) and *Alternaria* (Marin et al., 2013).

Most of the mentioned fungi are phytopathogenic species (Huffman et al., 2010), but a large number of important mycotoxin producers are also saprophytic species. Scientific research has mainly been focused on mycotoxins, which have a carcinogenic and/or toxic effect on humans and animals (Huffman et al., 2010). Toxicogenic

DOI: /10.5513/JCEA01/24.1.3704

fungi can produce one or more secondary metabolites, mostly *Fusarium* species, but not all metabolites are toxic (Solomon, 2011).

Mycotoxins of molds from the genus Fusarium

Fusarium species (Figure 1) are one of the largest producers of mycotoxins, the most important of which are trichothecenes, fumonisins and zearalenone (Vieira et al., 2020). They are pathogenic on cereals and other plant species and produce mycotoxins before or immediately after harvest (Pitt, 2000). They are among the most impactful pathogens which cause various diseases such as rotting of the root crown, cluster blight, root rot, etc. In recent years, *Fusarium* species have been studied for the mycotoxins they produce, due to their harmful effects on human and animal health (Hassan, 2019).

Figure 1. *Fusarium solani* (Source: Petrović, E.)

Trichothecenes

Trichothecenes are a group of 148 mycotoxins. They were first isolated from *Trichothecium roseum* and described by Freeman and Morrison in 1949 (Yazar and Omurtag, 2008). According to their chemical structure, they are divided into four groups, of which A and B are toxicologically more important. Group A includes T-2 toxin, HT-2 toxin, neosolaniol, scirpenol and its derivatives (diaceteoxyscirpenol-DAS, scirpetirol), while group B includes deoxynivalenol (DON), nivalenol and fuzarenone. In general, the most important trichothecene mycotoxins are DON, T-2 toxin, HT-2 toxin and diacetoxyscirpenol (Havranek et al., 2014). DON and nivalenol are among the many trichothecene mycotoxins produced by *Fusarium* species (Miller and Trenholm, 1996). Foroud and Eudes (2009) stated that DON is the most important mycotoxin associated with *Fusarium* head blight. DON shows great stability during storage/grinding and during food processing and cooking (Bosco and Mollea, 2012).

Trichothecenes are cytotoxic mycotoxins. Mycotoxins which belong to this group are strong inhibitors of protein synthesis (inhibit DNA synthesis), damage cell membranes, break down the nuclei of red bone marrow cells, etc. DON is the least toxic to domestic animals. Diacetoxycyrpenol occurs on corn and is very toxic to animals (Havranek et al., 2014).

Trichothecenes are mainly associated with cereals that are produced in regions with a mild climate in Europe, America and Asia, including wheat, barley, rye, oats, corn and rice (Yazar and Omurtag, 2008). Furthermore, presence has been recorded on soybeans, potatoes, sunflower seeds, peanuts and bananas, processed foods, mostly those produced from cereals (bread, breakfast cereals, noodles and beer) (Foroud and Edues, 2009). Trichothecenes can appear in the food chain through milk, meat and eggs of animals fed with the contaminated feed (He et al., 2010).

Fumonisins

Mycotoxins fumonisins are mainly produced by molds of the genus *Fusarium* (Marin et al., 2013). They were discovered in the 1980s as a result of years of research into a disease known as equine leukoencephalomalacia (Pitt et al., 1998). The best-known species are *F. moniliforme*, *F. verticilioides*, *F. proliferatum*, *F. dlaminii* and *F. napiforme* (Havranek et al., 2014). Fumonisins are also produced by *Alternaria alternata* (Chen et al., 1992) and *Aspergillus niger* (Huffman et al., 2010). There are at least 12 fumonisins, and the most important are from group B, designated as FB1, FB2 and FB3 (Marin et al., 2013;

Havranek et al., 2014). FB1, FB2 and FB3 mycotoxins are natural contaminants of cereals (CAST, 2003; Yazar and Omurtag, 2008).

Fumonisins are the most important corn mycotoxins, mostly occuring in warmer growing areas. Given that the species *F. verticilioides* and *F. proliferatum* can grow within in a wide temperature range, but only at relatively high water activity (aw > 0.9), FB toxins are formed on corn primarily during harvest in early stages of being stored (Marin et al., 2013).

Pitt (2000) states that fumonisins from corn are responsible for the esophageal cancer epidemic in Africa. Corn is the only significant source of this mycotoxin (Pitt and Hocking, 1997), but it can also be found in other foods such as rice, sorghum (CAST, 2003), in wheat noodles, curry, beer and corn-based cooking additives (Yazar and Omurtag, 2008). Fumonisin is produced when plants are under stress, or insects and mites have damaged the cereal grains (Havranek et al., 2014). Fumonisins are soluble in water, which is not the case with other mycotoxins (Havranek et al., 2014).

Zearalenone

Zearalenone is a mycotoxin produced by several *Fusarium* species; it is most often *Fusarium graminearum*, but also the species *F. culmorum*, *F. cerealis*, *F. esquieti*, *F. verticilioides* and *F. incarnatum* (Marin et al., 2013). Zearalenone is found in corn kernels, but also in oat and wheat kernels around the world. According to the pathological effect, it is similar to estrogen hormone. The mechanism of its toxic effect has not been explained (Havranek et al., 2014). Kuiper-Goodman et al. (1987) mention zearalenone as the cause of the premature puberty in children.

Moniliformin

It appears on corn kernels damaged by various insects and mites. It is produced by the species *F. proliferatum* and *F. subglutinans*. Its toxicity to humans is unknown, however it is extremely toxic to poultry. It is associated with fumonisin-contaminated food made from infected corn (Bianchini and Bullerman, 2014).

Mycotoxins of molds from the genus Penicillium

Penicillium fungi (Figure 2) produce blue-green powdery formations on corn grain, and their color may vary depending on the fungus species. Only certain fungi from the genus *Penicillium*, which live in corn kernels, produce mycotoxins. *Penicillium* species can produce 27 different mycotoxins, of which three are the most important such as: ochratoxin, patulin and citrinin (Havranek et al., 2014). In addition to the above, Hassan (2019) also includes rubratoxin, cyclopiazonic acid, tremorgenic, citreoviridin, luteoskirin, cyclochlorotin and regulosin as the most important mycotoxins. Certain species of the genera *Aspergillus* and *Penicillium* are pathogens on certain plant species, however these species appear as contaminants of products and food during drying and storage (Pitt, 2000). Magan and Aldred (2005) also state that *Penicillium* and *Aspergillus* most often do not attack the plant in the field but in the post-harvest phase.

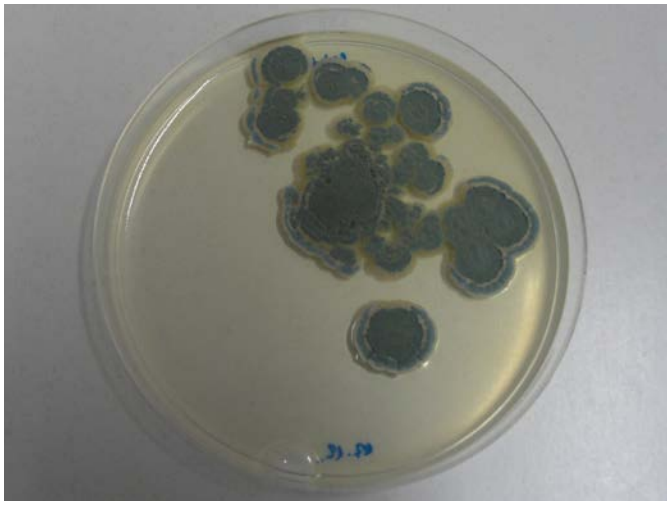

Figure 2. *Penicillium* sp. (Source: Petrović, E.)

Ochratoxin A

Ochratoxin A was discovered in 1965 in North Africa (Van der Merwe et al., 1965). It was isolated from maize (Shotwell et al., 1969). Ochratoxins, a large group of similar chemical compounds, and often citrinin, are produced by *Aspergillus ochraceus*, *Penicillium viridicatum*, and *Penicillium verrucosum* in areas with moderate climatic conditions (heat and humidity). Ochratoxin A is produced by certain strains of *Penicillium* fungi that are found in oat and barley grains before harvest, and can also be

found in corn grains in fields in some parts of the world (Havranek et al., 2014). *P. verrucosum* is responsible for contamination with ochratoxin A at lower temperatures, while *A. ochraceus* is more present in areas with a tropical climate (Battacone et al., 2010; Scudamore, 2003).

Ochratoxin A can be found in green coffee beans (Hassan, 2019). In practice, ochratoxin A is often found in corn and other grains (Havranek et al., 2014). Abrunhosa et al. (2010) and Scudamore (2003) state that ochratoxin A can be found in the following foods: corn, rice, wheat, barley, oats, rye, sorghum, millet, beer, baby food, packaged breakfast cereals, bread, broken grains and wheat, corn and rice bran.

Wine is important source of ochratoxin A for humans. Numerous works have proven the presence of toxins in wine, must and grape juice. The presence of toxins is explained by the fact that grapes are contaminated in the vineyard with different ochratoxicogenic species, primarily *Aspergillus* species, and the production of ochratoxin A increases with the stage of wine maturity (Cabañes et al., 2002; El Khoury et al., 2006). Adeyeye and Yildiz (2016) also report the occurrence of this mycotoxin in beer and wine. The species *A. carbonarius* is the prevalent of mycotoxins in raisins, wine and coffee (Pitt, 2000). Nawaz et al. (2017) report the occurrence of this mycotoxin in the following foods in addition to wine: fruit wine, flavored wine, grape-based juices, grape nectar, grape pomace, spices (black and white pepper, nutmeg, ginger, turmeric, dried fruit, chili powder, cayenne and paprika), liquid root extract, wheat gluten. Wolff et al. (2000) points out at the occurence of mycotoxins in many foods, including legumes, previously mentioned spices, meat and cheese. Ochratoxin A was found in garlic (Anjorin et al., 2020) (Figure 3) and spices with garlic (Iqbal et al., 2021).

Pitt (2000) states that people are infected with this mycotoxin through pork and bread made from barley or wheat infected with this toxin, nuts and green coffee beans. Hassan (2019) states that high concentrations of ochratoxin were also detected in pistachios.

The mechanism of pathological action of ochratoxin has not been sufficiently studied. Experiments have

Figure 3. Garlic infected with *Penicillium* mold (Source: Petrović, E.)

proven that ochratoxin causes reduced protein synthesis (Havranek et al., 2014). El Khoury and Atoui (2010) have reported several effects of ochratoxin on animals: nephrotoxic, hepatotoxic, neurotoxic, teratogenic and immunotoxicogenic. Ochratoxin is presumed to be carcinogenic to humans due to its proven carcinogenicity in animals (Huff et al., 1992).

Ochratoxin A has a high resistance to acidic environment and high temperatures and is characterized by generally high resistance and stability. It is very difficult to eliminate the ochratoxin A molecule: it partially degrades during cooking, and even after 3 hours of steam sterilization under pressure at 121 °C and 250 °C it does not degrade completely (Boudra et al., 1995). Certain processes in grain production, such as malt production, malt fermentation, production of bread and food products can lead to a decrease in the concentration of this mycotoxin in the final product (Baxter et al., 2001; Scott et al., 1995; Scudamore et al., 2003).

Patulin

It was discovered in 1943, related to the species *P. griseofulvum* and *P. expansum*. This mycotoxin was initially studied as a potential antibiotic, but more detailed studies have shown its toxic properties (Baert et al., 2007; Birkinshaw et al., 1943). Patulin causes nervous and digestive disorders, and it is produced by the fungus *P. expansum*, which can grow at temperatures from -2 °C to 35 °C (Havranek et al., 2014).

Moake et al. (2005) and de Souza Sant' Ana et al. (2008) have stated that this mycotoxin could been isolated from various types of fruits and vegetables or from pasteurized and unpasteurized foods, but in the food industry it is the most common contaminant of apples (Figure 4) and apple products. FAO (2003) emphasizes the importance of maintaining favorable storage conditions and removing infected fruit, considering that patulin most often occurs in damaged moldy fruit. In order to improve the storage conditions, it is recommended to use various treatments such as the application of sanitary means, natural or biological preparations (Chen et al., 2004), the use of polyethylene packaging with or without a controlled atmosphere during storage and transport (Moodley et al., 2002). Patulin can be removed from the juice by filtering through granulated activated carbon (Kadakal and Nas, 2002), and the proportion can be reduced by adding various additives (ascorbic acid, thiamine hydrochloride, pyridoxine hydrochloride and calcium pantothenate) (Yazici and Velioglu, 2002).

Symptoms of the animal poisoning include damage to the liver, spleen and kidneys and toxicity to the immune system. Nausea and gastrointestinal disturbances occur in humans. Patulin is considered genotoxic, although its potential carcinogenicity has not yet been proven (WHO, 2018).

Citrinin

Citrinin is a nephrotoxin produced mainly by P. citrinum, *P. expansum* and *P. verrucosum* at temperatures from 15 °C to 37 °C (the optimal production temperature is 30 °C) (Havranek et al., 2014). Citrinin is associated with yellow rice disease in Japan and has a nephrotoxic effect on animals (Bennett and Klich, 2003). It has been found in many foods (wheat, rice, corn, barley, oats, rye and foods colored with "Monascus" pigment), but its effect on human health is unknown (Ashiq, 2015; Bennett and Klich, 2003).

Mycotoxins of molds from the genus Aspergillus

The most important mycotoxins produced by species of the genus *Aspergillus* are aflatoxins, ochratoxin A, cyclopiazonic acid and sterigmatocystin. *A. paraciticus* is among the most common species that produces mycotoxins, and *Aspergillus* genus (Figure 5) can contaminate corn and other cereals in the fields and in warehouses. Molds from the genus *Aspergillus* are spread all over the world, and are most often found in warm stressors.

Figure 4. Apple infected with *P. expansum* (Source: https://pesticideguy.org/2014/01/16/toxins-appear-in-apple-juice-due-to-fungicide-cancellations)

Figure 5. *Aspergillus* sp. (Source: Petrović, E.)

DOI: /10.5513/JCEA01/24.1.3704

Environmental temperature above 25 °C and drought are mentioned as stress for *A. flavus*, which then produce large amounts of aflatoxin (Havranek et al., 2014). Hassan (2019) states that more than 40 *Aspergillus* species produce more than 60 mycotoxins.

Aflatoxin

Aflatoxins are produced by the molds *A. flavus*, *A. parasiticus*, *A. nominus* (Havranek et al., 2014), *A. pseudotamari*, *A. pseudocaleatus*, *A. pseudonomius*, *A. versicolor*, *A. fumigatus*, *A. niger*, *A. carbonarius*, *A. ochraceus*, *A. terreus*, *A. sydowii*, *A. wentii*, *A. ustus* and *A. candidus* (Hassan, 2019). Aflatoxins cause liver inflammation, and deterioration, and malignant tumors, and are considered a strong liver carcinogen for humans and various animal species (Havranek et al., 2014). They are the best known and best researched mycotoxins. They were first discovered in the 1960s after more than 100,000 people died from food poisoning that contained contaminated peanuts (Blount, 1960; CAST, 1989). They belong to the most toxic mycotoxins produced by certain fungi that grow in the soil, contaminating vegetation, hay and grain; cereals, oilseeds, herbs and nuts (WHO, 2018). Peanuts, corn and cottonseed are the three most important crops infected by these molds (Pitt, 2000). They can be found in the milk of animals fed with contaminated feed (WHO, 2018), cheese (Barbiroli et al., 2007; Brackett and Marth, 1982), dried fruits and peas (Hassan, 2019). In addition to the above, they can also be found in vegetable oils, cocoa and rice (JECFA, 1998; ROC, 2003).

Pitt (2000) points out at the existence of four main naturally produced aflatoxins, namely B1, B2, G1 and G2. "B" and "G" are the designation that refer to the blue and green fluorescent colors produced by these components under UV light on the thin-layered chromatographic plates, while the numbers 1 and 2 indicate the minor and major components, respectively (Pitt, 2000). Aflatoxin B1 is being considered the greatest human carcinogen (IARC, 1993).

Contamination with aflatoxins can occur before and after the harvest. However, the highest concentration is created if food is being storaged in unfavorable conditions, with increased humidity and high temperature, which promote the development of mold. The level of contamination depends on the stress experienced by the plant, temperature, water activity, genotype, cultivar and storage conditions. However, appropriate post-harvest treatments and storage in dry and cool conditions can prevent the contamination (Moss, 2002; Wilson and Payne, 1994). Aflatoxins are produced by fungi at a temperature of 12 to 40 °C, at a pH of 3.5-8. *A. flavus* can produce aflatoxin and cyclopiazonic acid simultaneously, at a water activity of 0.996 AwA. Aflatoxins are primarily hepatotoxic. Aflatoxins cause coagulopathies similar to those caused by anticoagulant rodenticides (coumarin, bromadiolone, pindone). They inhibit the activity of DNA-polymerase and RNA-polymerase with the consequent inhibition of the synthesis of nucleic acids, from where the cancerogenic effects on an organism originate from. Aflatoxins also have an immunosuppressive effect (Havranek et al., 2014). In humans, they cause vomiting, abdominal pain, edema, coma, convulsions, and death (Mwanda et al., 2005).

Cyclopiazonic acid

Cyclopiazonic acid is primarily produced by the species *A. flavus* and some species of fungi from the genus *Penicillium*. The increased concetrations of the cyclopiazonic acid leads to necrosis in most organs (Hassan, 2019). Cyclopiazonic acid is naturally found in corn, peanuts, millet (a cause of human poisoning in India) (Bianchini and Bullerman, 2014), rice (Hayashi and Yoshizawa, 2005), tomatoes and tomato products (da Motta and Soares, 2010), cereals, cooked ham, cheese, fruit and nuts (Gill-Serna et al., 2014). Bianchini and Bullerman (2014) points out at the impact of mycotoxins on weight loss, dehydration, etc. in animals.

Sterigmatocystin

Sterigmatocystin is a metabolite of the molds *A. versicolor*, *A. nidulans* and *Bipolaris* species. They have a hepatotoxic and hepatocarcinogenic effect (Hassan, 2019). Jurjević et al. (2013), Visagie et al. (2014) and

Jakšić Despot et al. (2017) have also mentioned other species: *A. amoenus*, *A. creber*, *A. cvjetkovicii*, *A. fructus*, *A. griseoaurantiacus*, *A. hongkongensis*, *A. jensenii*, *A. pepii*, *A. protuberus*, *A. puulaauensis*, *A. subversicolor*, *A. tennesseensis* and *A. venenatus*.

This mycotoxin can be found in many foods and foodstuffs, as well as building materials, etc. (Viegas et al., 2015). It was found in rice (Sawane and Sawane, 2014; Mo et al., 2015; Rofiat et al., 2015; Bertuzzi et al., 2017), bread (Veršilovskis and Bartevičs, 2012), wheat bran, (Tančinová and Labuda, 2009), wheat (Veršilovskis et al., 2008; Mo et al., 2015;), corn (Warth et al., 2012; Mo et al., 2015;), peanut products (Warth et al., 2012), peanut seeds (Youssef et al., 2008), coffee beans (Bokhari and Aly, 2009; Culliao and Barcelo 2015), beer (Veršilovskis et al., 2008) and cheese (Veršilovskis et al., 2009).

It is a possible cause of cancer in humans (McConnell and Garner, 1994) and has an immunotoxic and immunomodulating effect (Liu et al., 2014).

Gliotoxin

Gliotoxin leads to damage to the respiratory organs. It is produced by the molds *A. flavus*, *A. fumigantus*, *A. niger*, *A. terreus*, *Eurotiun chevalieri* and *Neosartorya pseudofischeri* (Hassan, 2019). It has an immunosuppressive, genotoxic and cytotoxic effect (Nieminen et al., 2002; Upperman et al., 2003). It is a contaminant of corn, corn products, wheat, barley, oats, meat and other food for humans and animals (Pena et al., 2009).

Mycotoxins of molds from the genus Claviceps purpurea

Ergotism is the poisoning of the domestic animals, poultry and humans with toxins produced by the parasitic fungus *Claviceps purpurea*. Poisoning is manifested by tissue necrosis and nervous disorders (convulsions). It parasitizes mainly on the rye, but it can also appear other cereals, e.g. oats, barley, wheat and some grasses. *C. purpurea* fungal spores infect flowering plants, settle in the fruiting body and destroy the seed embryo. Thus, instead of a grain, a dark purple hard seed (sclerotium) resembling a banana fruit develops 1-3.5 cm in size, which contains a lot of toxic substances known as ergot-alkaloids (Hassan, 2019).

In total 40 related toxins produced by *C. purpurea* have been identified. Some of them are used in the pharmaceutical industry as medicines (ergotamines). The pathological effect of ergot alkaloids is localized on the vegetative nervous system. The periods high in humidity favor the development of the fungus *C. purpurea* on the ears of cereals and grasses. They are spread by wind, trough contaminated soil and cereal seeds. All kinds of animals and humans can suffer from rye head poisoning. People get poisoned by consuming foods of plant origin that consist of cereal grains mixed with sclerotia of the causative agent of ryegrass. Today, human poisonings are rare because less rye is consumed, and rye grain separation procedures have improved (Hassan, 2019).

Mycotoxins of "black" mold species

This group includes *Alternaria* toxins, macrocyclic trichothecenes and *Trichoderma* toxins. The most relevant secondary metabolites with toxic effects produced by *Alternaria* (Figure 6) species are alternariol (AOH), alternariol monomethyl ether (AME) and tetramic and tenuazonic acid derivatives. AOH and AME are also produced by *Stagnospora nodorum* and *Phomopsis species* (Hassan, 2019).

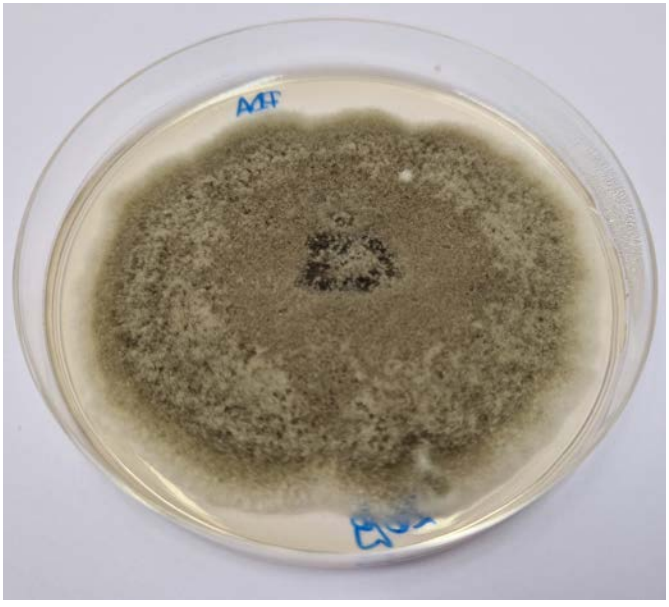

Figure 6. *Alternaria alternata* (Source: Petrović, E.)

AOH and AME can be found in cereals and cereal products, tomatoes, fruits such as apples and berries (Escrivá et al., 2017), apple juice, tomato products,

DOI: /10.5513/JCEA01/24.1.3704

sunflower seeds, canola, flax seeds (Ostry, 2008; Logrieco et al., 2009) and Canadian lentils (Ostry et al., 2004). Tenuazonic acid has been found in beer and other cereal products (Siegel et al., 2010; Asam et al., 2012).

Analysis of mycotoxins in food and drink

The determination of mycotoxins in food and beverages is carried out by methods that include the following procedures: sampling, homogenization, extraction and purification, detection and quantification performed by instrumental and non-instrumental methods (Whitaker, 2003; Pereira et al., 2014; Shephard, 2016).

Methods for the analysis of mycotoxins in food are: chromatography, immunochemical methods, rapid tests (LFD test, "dipstick"), infrared spectroscopy, capillary electrophoresis, application of molecularly imprinted polymers and biosensors, fluorescent polarization and electronic nose device (Alshannaq et al., 2017).).

Chromatography is the most commonly used method for the analysis of mycotoxins in food and animal feed (Shephard, 2016). Gas chromatography, combined with mass spectrometry and fluorescence detectors, is routinely used for analysis. However, other chromatographic methods are also used depending on the sensitivity and specificity of the analysis (Pereira et al., 2014; Anfossi et al., 2016). The best-known and most frequently used immunochemical method for the analysis of mycotoxins is the ELISA (enzyme-linked immunosorbent assay) (Pereira et al., 2014). The principle of the method is based on the competition between the mycotoxin, which acts as an antigen, and the corresponding antibody labeled with the enzyme of the toxin (Turner et al., 2009; Pittet, 2005). Rapid methods of determination include the application of the LFD (eng. lateral-flow device) test and the "dipstick" test. The LFD test is based on the competition, where a labeled antibody is used as a signaling reagent (Krska and Molinelli, 2009). The "Dipstick" test is similar to the ELISA method and is used for the detection of individual mycotoxins in food (Schneider et al., 1995; Maragos and Busman, 2010). Infrared spectroscopy is an optical method that combines infrared light and principal component analysis for the detection and quantification of mycotoxins, and represents a fast and non-destructive method for detecting mycotoxins in cereals (Pettersson and Aberg, 2003). Capillary electrophoresis is an instrumental technique that separates different components based on their electrochemical potential using fluorescence and UV absorbance (Cornelli and Maragos, 1998). More and more popular detection methods are the application of molecularly imprinted polymers (imitates antibody-antigen interaction), biosensors, fluorescent polarization (measures the interaction between fluorescently colored antigen and specific antibody), and electronic nose (variant of gas chromatography) as fast and cheap methods of detecting mycotoxins in food (Vasapollo et al., 2011, Logrieco et al., 2005; Maragos, 2006; Keshri and Magan, 2000).

Reducing the risk of mycotoxins

WHO (2018) points out that fungi that produce mycotoxins live on different crops and in different foods and can penetrate deep into the food and do not just grow on its surface. Mold generally does not grow in properly dried and stored food, therefore adequate drying and maintenance of dry conditions, i.e. adequate storage, is an effective measure against the formation of fungi that produce mycotoxins. They also emphasize the importance of packaging control and avoiding damage of stored food.

Timely harvesting reduces the risk of mycotoxin contamination. The occurence of mycotoxins largely depends on temperature, humidity, water activity, pH value and oxygen concentration, i.e. on the same conditions that affect the growth of toxicogenic fungi. Moisture and temperature are the two factors that have a crucial influence on the development of the fungus and the synthesis of toxins (Bryden, 2007; Paterson and Lima, 2010).

During the post-harvest period, moisture and temperature control of stored products can reduce the risk of developing toxicogenic fungi and their mycotoxins (Bryden, 2007). All grains must be dried to a minimum of 15% moisture (Hassan, 2019). Fox and Howlett (2008) state that by avoiding water stress, minimizing insect

attacks, applying good agricultural practices, during and after harvesting, the risk of mycotoxin infection can be reduced. Infected plant residues must be removed from the field. Methods such as crop rotation, sowing preparation, optimal sowing/planting date, and irrigation and fertilization reduce mycotoxin infection and spread (van Zyl et al., 2015). By reducing the concentration of oxygen, using additives (ammonia, propionic acid, microbiological preparations and enzymes for silage), appropriate silo size, cleaning the grain container, adequate moisture, aeration, avoiding the consumption of moldy food, using inhibiting substances (salts, propionic acid, acetyl acid) and others, we can greatly reduce the risk of food contamination with mycotoxins and the occurrence of mycotoxicosis, emphasizes Hassan (2019).

The risk of mycotoxin occurrence depends not only on the degree of contamination but also on the length of the exposure. Testing food for one mycotoxin cannot show the accurate picture as other mycotoxins migth be present. Mycotoxins have a synergistic effect, so the combination of several species has a devastating effect (Hassan, 2019).

The removal of mycotoxins from food can be done by washing, aspiration, gravity separation (removing weed seeds, small grains, etc.), heating (the proportion of mycotoxins is reduced, but not completely, since mycotoxins are thermostable), irradiation, etc.

Bullerman and Bianchini (2007) state the importance of the temperature treatments on the concentration of mycotoxins in foods. Foods exposed to high temperatures (baked, fried foods above 150 °C) have a lower concentration of mycotoxins.

Treatment with chemicals, including sodium bisulfate, ozone and ammonia, acids and bases, allows control of fungal growth and mycotoxin synthesis in stored grain (Bozoglu, 2009; Magan, 2006; Magan and Aldred, 2007). In recent years, the development of mycotoxicogenic fungi has been controlled by the use of plant preparations (extracts and essential oils) as environmentally acceptable fungicides (Nguefacka et al., 2004; Reddy et al., 2010; Thembo et al., 2010).

CONCLUSION

Mycotoxin contamination is an economic problem for animal breeders and the food industry. The presence of mycotoxins in food reduces the food quality, including nutritional and protein value. The occurrence of mycotoxins in broken grain can be 30 to 500 times higher than in the whole grain (Hassan, 2019).

It is estimated that about 25% of agricultural crops are infected with fungi, and as a result, food can be contaminated with mycotoxins before or after the harvest. Mycotoxins present in food vary from country to country depending on the type of crop, agricultural practices and climatic conditions (Bryden, 2007).

Some authors believe that there is a strong possibility that mycotoxins are the cause of poorer health in people in parts of the world with a warmer climate (Pitt, 2000).

Therefore, it is necessary to adhere to good hygiene and production practices from field to table. It is necessary to monitor the condition of crops, plant/sow healthy planting material, treat crops on time, control production processes, but also storage conditions in order to minimize the risk of contamination of food with fungi and their mycotoxins.

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