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Review

Mycotoxins in foods, from the field to the plate: a review

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Abstract

Mycotoxins are secondary metabolites produced by some fungal species, mainly from the genera *Alternaria, Aspergillus, Fusarium*, and *Penicillium*. Mycotoxins can be found in raw materials and processed foods. High intake of mycotoxins in short time periods will generate outbreaks of mycotoxicosis distinguished by physical discomfort or even death. Chronic consumption of mycotoxins can cause several important illnesses. Due to the substantial health risk of mycotoxin intake, several organisations have recommended the maximum allowable limits in foods. Since differences in the values suggested across organisations affect the risk of populations ingesting these compounds, the criteria must be unified. Mycotoxins are generally highly thermostable. Operations commonly applied during food processing such as frying and roasting have variable effects in reducing the mycotoxin content. The use of probiotics to transform mycotoxins into minor toxic compounds is a promising alternative reduction measure. The complete elimination of mycotoxins in foods appears practically impossible. Therefore, good agronomic practices are essential to avoid the growth of mycotoxin-producing fungi in raw materials. Global climate change is a relevant issue due to the changes in rainfall, humidity, and temperature patterns worldwide could stimulate the growth of fungi in broader regions, thus increasing the risk of mycotoxin presence in foods and subsequent consumption. Therefore, increasing research and development in innovative methods for the elimination or reduction of mycotoxins in foods is essential.

Introduction

Mycotoxins are low molecular weight compounds synthesised in the fungal hyphae and conidia of several species, principally the genera *Alternaria, Aspergillus, Fusarium*, and *Penicillium* (Jajić *et al.,* 2019). Although more than 450 mycotoxins are known, the most important ones in food products on the basis of toxicity, residual presence in foods, and wide geographic distribution are aflatoxins, ochratoxins, trichothecenes, fumonisins, and zearalenone (Gruber-Dorninger *et al.,* 2019; Benkerroum, 2020). These compounds are produced as a defence under stress conditions during secondary metabolism by certain fungal species from the aforementioned genera. The uptake of mycotoxins can be oral, nasal, or transdermal. Acute or chronic ingestion produces adverse effects on human and animal health, and may result in death (Yard *et al.,* 2013).

The presence of mycotoxins in foods depends on the care taken during handling since contamination can occur before or after harvest (Kaushik, 2015, Ozturkoglu-Budak, 2017). Fungal species of the genera *Fusarium* and *Aspergillus* are plant pathogens that produce mycotoxins in the field during plant growth. However, some species of *Penicillium* and *Aspergillus* could also colonise and produce mycotoxins in products during storage (Panasiuk *et al.,* 2019; Agriopoulou *et al.,* 2020). Importantly, the presence of fungi is not always indicative of the presence of mycotoxins, and fungal elimination does not ensure that mycotoxins are eliminated (Schmidt *et al.,* 2018). The concentrations of mycotoxins in foods can differ across countries and even regions because of crop varieties, agronomic practices, and prevailing climatic conditions (Eskola *et al.,* 2019; Gruber-Dorninger *et al.,* 2019). Unfortunately, even the best agricultural practices, postharvest handling, storage, and food processing cannot ensure mycotoxin absence, and thus their complete eradication (Schmidt *et al.,* 2018).

Mycotoxins can be present in low moisture foods such as nuts (Wang *et al.,* 2018b), dried fruits (Fanelli *et al.,* 2017; Ozturkoglu-Budak, 2017), cereals (Habschied *et al.,* 2019; Spanic *et al.,* 2020), cereal products (Abia *et al.,* 2017; Generotti *et al.,* 2017), oilseeds (Mohammed *et al.,* 2018), feeds (Lee *et al.,* 2015), and fermented products such as beer

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(Peters *et al.,* 2017; Mastanjević *et al.,* 2018) and some types of tea (Wang *et al.,* 2018a). Mycotoxin presence is also observed in foods of animal origin such as dairy products (Anelli *et al.,* 2019; Vaz *et al.,* 2020), meat (Al Khalaileh, 2018; Meucci *et al.,* 2019), eggs (Jurisic *et al.,* 2019), and breast milk (Cantú-Cornelio *et al.,* 2016; Arcella *et al.,* 2017); fruits such as apples (Li *et al.,* 2020), fruit juices (Kalagatur *et al.,* 2018; Pallarés *et al.,* 2019) and wines (He *et al.,* 2019); and vegetable oils (Hidalgo-Ruiz *et al.,* 2019).

The presence of mycotoxins in foods and feeds causes substantial economic losses estimated to range from hundreds of millions to billions of dollars per year (Pinotti *et al.,* 2016; Ozturkoglu-Budak, 2017). The most important economic impact is associated with human health in developing countries (Alberts *et al.,* 2016; Adeyeye, 2019), although few comprehensive studies have investigated the occurrence of most mycotoxins in these countries (Eskola *et al.,* 2019). Mycotoxins in feeds pose a risk to human health, because when animals ingest them, these compounds can accumulate in organs or muscle (Al Khalaileh, 2018), excreted through the milk, or even transferred to eggs (Flores-Flores and González-Peñas, 2018; Jurisic *et al.,* 2019).

Most mycotoxins are thermostable compounds (170 to 350°C). Hence, they resist the thermal treatments regularly applied during food processing such as baking, roasting, frying, and pasteurisation (Vidal *et al.,* 2014; Vaz *et al.,* 2020). For example, aflatoxins require temperatures above 150°C to begin to degrade, but they require heating to at least 216°C for 35 min in the absence of matrix interference to completely decompose (Gbashi *et al.,* 2019). Deoxynivalenol is highly stable, resisting temperatures from 170 to 300°C (Generotti et al., 2017). Fumonisins require baking at 200°C for 15 min for complete degradation in a corn matrix (Gbashi *et al.,* 2019). Ochratoxin A is highly stable and resistant to acidic environments, and it degrades at temperatures exceeding 210°C (Gbashi *et al.,* 2019). Some products of mycotoxin degradation still maintain some level of toxicity (Kószegi and Poór, 2016; Pierron *et al.,* 2016).

Mycotoxins most relevant to human health

The effects of mycotoxin consumption on human health can be acute or chronic. Acute effects result from the intake of substantial amounts of mycotoxin in short period of time. These effects have been associated with abdominal pain, severe liver disease, vomiting, and even mortality rates as high as 40% in countries such as Asia and Africa during mycotoxicosis outbreaks (Yard *et al.,* 2013). Chronic effects of mycotoxin consumption result from the intake of moderate doses for long period of time, and are also relevant to health (Kovalsky *et al.,* 2016). These effects include reduced growth, immunosuppression, development of cancer, neurotoxicity, nephrotoxicity, mutagenicity, and cytotoxicity (Kószegi and Poór, 2016; Benkerroum, 2020). Pharmacokinetics studies have indicated that chronic mycotoxin intake at levels exceeding those that the organism can excrete might cause their accumulation in the body and long-term effects on health. These effects can drive the development of diseases that might be confused with other developmental factors, such as dietary or physiological factors (Ojuri *et al.,* 2018).

The chemical structures of the most studied mycotoxins are shown in Figure 1. Aflatoxins, ochratoxins, trichothecenes, fumonisins, and zearalenone are the most hazardous mycotoxins to human health (Braun *et al.,* 2018; Benkerroum, 2020). Due to their acute and chronic toxic effects, their maximum contents allowed in foods and feeds are regulated by many countries worldwide (Kovalsky *et al.,* 2016; Gruber-Dorninger *et al.,* 2019). The principal genera of fungi that produce these regulated mycotoxins are *Alternaria, Aspergillus, Fusarium*, and *Penicillium*. However, fungi can produce hundreds of secondary metabolites whose toxicity has not been extensively analysed or associated with epidemiological outbreaks; given their hazard potential, some are considered emerging mycotoxins (Kovač *et al.,* 2018; Panasiuk *et al.,* 2019). An example of this type of compound is fusarenon-X, a non-regulated mycotoxin, which has been poorly studied but its presence in foods derived from cereals has been reported in Asia. According to Alassane-Kpembi *et al.* (2017), this emerging mycotoxin has higher toxicity than deoxynivalenol (a regulated trichothecene), inducing severe intestinal inflammation; however, confirmative reports are needed.

Aflatoxins are produced by several species of the genus *Aspergillus*, among which *A. flavus* and *A. parasiticus* are prevalent (Mastanjević *et al.,* 2019). The effects of aflatoxins on animals have been demonstrated to be carcinogenic, teratogenic, hepatotoxic, and mutagenic (Alim *et al.,* 2018). These compounds include B_1, B_2, G_1, and G_2 (AFB_1, AFB_2, AFG_1, and AFG_2) types, among which AFB_1 is considered the most potent (Adeyeye, 2019; Benkerroum, 2020). When AFB_1 is consumed, a portion of it is metabolised and biotransformed into aflatoxin M_1 (AFM_1) in organisms. AFM_1 can be excreted through the mammary glands, thus explaining why it is detected in milk samples (Vaz *et al.,* 2020).

Ochratoxins are economically important

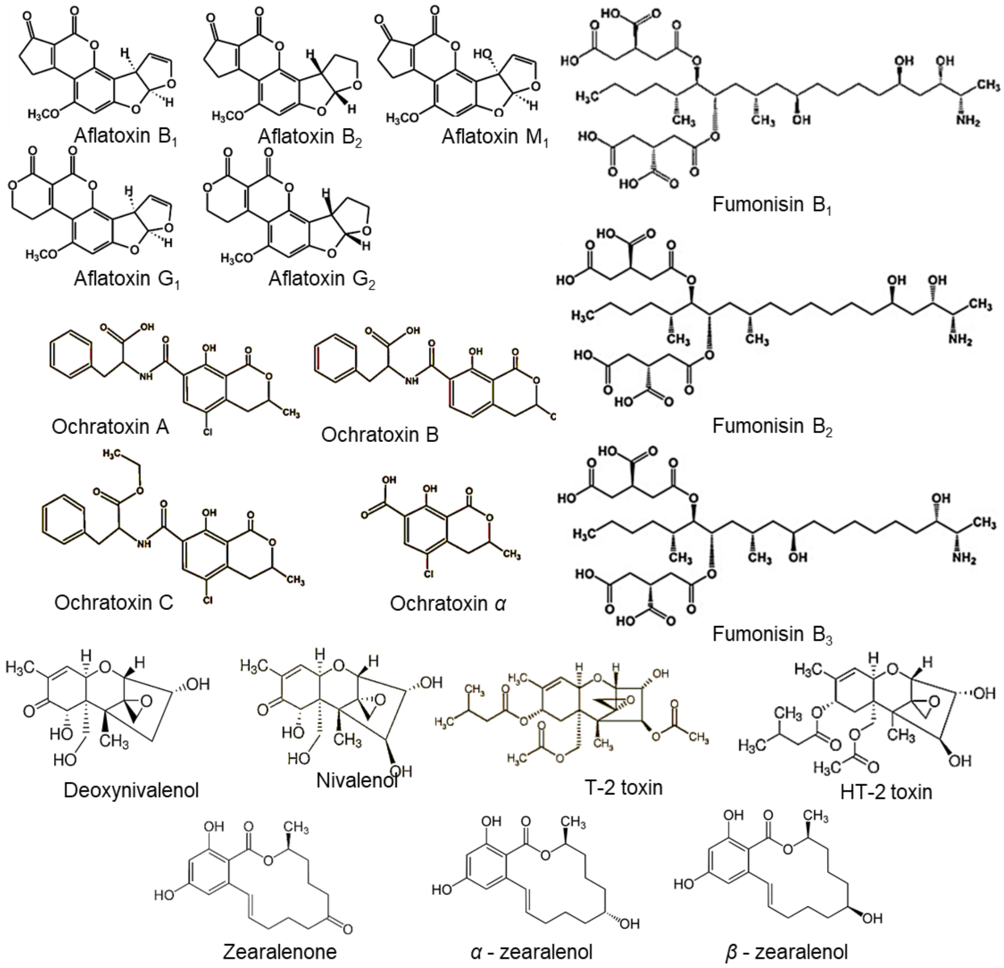

Figure 1. Chemical structure of some of the most studied mycotoxins.

because of their presence in high-value commodities, such as coffee and wine (Kószegi and Poór, 2016). *Aspergillus ochraceus, A. carbonarius, A. niger*, and *P. verrucosum* are the main species responsible for ochratoxin production (Bui-Klimke and Wu, 2015). Ochratoxins exist in three compositional configurations (A, B, and C) in nature, but OTA is most frequently found in foods. Since animal studies have demonstrated that OTA produces teratogenic, hepatotoxic, neurotoxic, immunotoxic, and carcinogenic effects, it is considered the most hazardous analogue of the group. Human exposure to OTA is evidenced by the frequent detection of this compound and its major metabolite, ochratoxin α (OT α), in blood, urine, and breast milk samples of people in diverse regions worldwide (Ali *et al.,* 2017, 2018; Šarkanj *et al.,* 2018). Elevation of OTA in certain areas worldwide has been associated with a high incidence of testicular cancer, upper urinary tract and oesophageal tumours (Kószegi and Poór, 2016; Pierron *et al.,* 2016), Balkan endemic nephropathy, and chronic interstitial nephropathy (Bui-Klimke and Wu, 2015).

Trichothecenes are mycotoxins produced by some species of *Fusarium* (Mastanjević *et al.,* 2019). These metabolites exist in four chemical conformations (groups A - D). The A and B groups are most frequently found in foods. Group A includes T-2 and HT-2, whereas deoxynivalenol (DON), nivalenol, and fusarenon-X are in group B (Arcella *et al.,* 2017; Agriopoulou *et al.,* 2020). DON is the most studied mycotoxin among the trichothecenes. It is produced mainly by *F. graminearum*, a mould that affects crops such as wheat, barley, and corn. Consequently, DON is frequently found in grains, cereals, and foods made from them, such as breakfast cereals and bakery products (Stadler *et al.,* 2019). It is highly soluble in water and polar solvents (Generotti *et al.,* 2017). Its acute effects mainly alter the immune system and the gastrointestinal tract causing diarrhoea, vomiting, abdominal pain, fever, and headaches (Pierron *et al.,* 2016).

Fumonisins are produced primarily by fungi such as *F. verticillioides* and *F. moniliforme*. A total of 28 fumonisins have been identified and grouped into four categories (A, B, C, and P). Fumonisins B_1, B_2, and B_3 are frequently found in foods, with FB_1 considered the most important due to it having the highest abundance (Alberts *et al.,* 2016; Eskola *et al.,* 2019). These mycotoxins have been found in cereals such as corn, rice, and sorghum, as well as processed foodstuffs such as peanut butter, wheat, pasta, and beer (Peters *et al.,* 2017; Ojuri *et al.,* 2018). The harmful effects of fumonisin consumption are associated with their carcinogenicity, liver, and kidney toxicity in animals (Voss *et al.,* 2017; Bordini *et al.,* 2019). Some studies have associated elevated incidence of oesophageal cancer in regions of the world such as the Linxian and Cixian counties in China, with high levels of fumonisins observed there (Zhang *et al.,* 1997).

Zearalenone is a widely distributed mycotoxin (Chang et al., 2020), produced by several *Fusarium* species such as *F. graminearum, F. culmorum,* and *F. verticillioides* (Mally *et al.,* 2016). It is produced in the field, and therefore is often detected in corn grains and products such as flour and oil (Song *et al.,* 2018; Chang *et al.,* 2020). It is also found in other cereal grains such as rice, sorghum, and rye, as well as their processed products such as flours, baked foods, beer (EFSA, 2011; Mally *et al.,* 2016; Ali and Degen, 2019), as well as in edible and medicinal herbs (Sun *et al.,* 2018). Zearalenone is primarily notable for its estrogenic effects (Chang *et al.,* 2020). It is generally stable during cooking, resisting temperatures up to 150°C, except under alkaline conditions or during extrusion cooking (EFSA, 2011). Its consumption has been evidenced by its detection and that of its metabolites (α-zearalanol, β-zearalanol, α-zearalenol, β-zearalenol, and zearalanone residues) in pasteurised milk samples (Jiang *et al.,* 2018) and in urinary samples of peoples from several regions of the world (Ali and Degen, 2019; Gratz *et al.,* 2020).

The presence of hazardous fungal secondary metabolites in foods includes the so-called emerging and masked mycotoxins. These comprise a considerable number of compounds, some of which are precursors of well-known and regulated mycotoxins, whose negative effects on human health are currently inconclusive (Stanciu *et al.,* 2017; Spanic *et al.,* 2020). A risk of consumption of emerging mycotoxins by humans is suspected because their toxic effects have been demonstrated in laboratory animals or *in vitro* studies (Ali *et al.,* 2018; Hamad *et al.,* 2018). Emerging mycotoxins such as enniatins, beauvericin, moniliformin, fusaproliferin, fusaric acid, culmorin, butenolide (produced by *Fusarium*), sterigmatocystin, and emodin (produced by *Aspergillus*), as well as alternariol, alternariol monomethyl ether, tenuazonic acid, and altertoxins (produced by *Alternaria*) have been found in foods and feeds, thus compromising safety (Panasiuk *et al.,* 2019; Agriopoulou *et al.,* 2020).

Masked mycotoxins are conjugated forms of regulated mycotoxins. These also constitute a threat, because most of them can be transformed and compartmentalised in plants as part of the plant´s metabolic defence mechanisms against xenobiotics, and are later released in consumer organisms (Kovač *et al.,* 2018; Mastanjević *et al.,* 2019). Some of these compounds are bound to carbohydrates or proteins, thus making their extraction and quantitation difficult (Kovalsky *et al.,* 2016; Šarkanj *et al.,* 2018); consequently, their content in food may be underestimated. Emerging and masked mycotoxins may exert toxic effects by themselves, although they can also be metabolised and converted into their original forms in the consumer body. Some laboratories have reported that several metabolic products of regulated mycotoxins (e.g., modified forms of zearalenone and DON) might generate more damage in the organism than their precursors (Kovalsky *et al.,* 2016; Mally *et al.,* 2016). Since further studies are needed to demonstrate the risk of consumption of emerging and masked mycotoxins, they are currently not regulated (Panasiuk *et al.,* 2019; Kovač *et al.,* 2020).

Limits of mycotoxins in food

Maximum recommended concentrations for several mycotoxins have been established in foods and feeds in more than 100 countries (Matumba *et al.,* 2017). The maximum levels of mycotoxins allowed in products are based on their toxicity, the type of food, and the potential consumers of that food. The Scientific Committee on Food and the European Food Safety Authority from the Commission Regulation of the European Community established recommendations, and periodically review them according to scientific reports and recent studies (EC, 2006).

The Codex Alimentarius Commission of the Food and Agriculture Organisation aims to establish maximum levels of mycotoxins and aflatoxins, particularly for international application. The United States was one of the pioneers in regulating aflatoxin levels in food. The Food and Drug Administration (FDA) is the organisation that sets regulations and supervises food safety and consequently determines the maximum concentrations of mycotoxins allowed in food in the United States. According to the FDA, the maximum amount of aflatoxins permitted in food is 20 µg/kg. The FDA regulations have been adopted in 17 Latin American nations that trade with the United States; thus, the highest concentration of aflatoxins in cereals is equal to that set by the health authorities from the United States (van Egmond and Jonker, 2004).

The maximum recommended concentrations of mycotoxins in the United States are usually higher than those allowed in Europe (Al-Taher *et al.,* 2017). For example, the maximum amount of aflatoxins in food tolerated by the FDA is 20 µg/kg (FDA, 2000), whereas in Europe, the maximum is 10 µg/kg. Similarly, while the maximum concentration for AFM_1 in milk for adult consumption is 0.5 µg/kg according to the FDA, in the European Union, the allowable limit is ten times lower (van Egmond and Jonker, 2004). This is a relevant issue, because some foods unacceptable for consumption in the European Union do not exceed the limits indicated by the FDA. Thus, a report has found that 78% of tested infant food samples showed contamination with at least one mycotoxin, and although the levels were permissible according to FDA limits, approximately 30% had concentrations above the European limits, and therefore were unacceptable for consumption (Al-Taher *et al.,* 2017).

Table 1 shows the maximum recommended consumption limits of some of the most studied mycotoxins according to the European Commission (EC, 2006) and the European Food Safety Authority (EFSA, 2014). The maximum limits have been determined according to scientific evidence of their occurrence, concentration in food, and toxicity (EC, 2006). Temporary upper limits are stated for some mycotoxins, for which, limited information is available. The maximum tolerable limits of intake are constantly revised when more data are available (EC, 2006). Tolerable limits of intake are not applicable to aflatoxins, because their carcinogenic effects at concentrations as little as < 1 ng/kg/bw/d have been demonstrated; therefore, their consumption should be as low as reasonably achievable (Matumba *et al.,* 2017). Of note, children are more susceptible than adults to the effects of consuming mycotoxins because of their lower body mass, higher metabolic rate, and lower detoxification capacity (Arcella *et al.,* 2017; Oueslati *et al.,* 2018), Thus, the maximum mycotoxin content in foods for infants is considerably lower than that allowed in products generally consumed by adults (EC, 2006; EFSA, 2011).

Mycotoxin detection is associated with the presence of certain fungal species. For example, when *Aspergillus* spp. are detected, aflatoxins are expected to be found in the products. Since *Aspergillus* spp. are frequently found in foods such as tree nuts, groundnuts, oilseeds, dried fruits, spices, cereals, and their products, their mycotoxins are also expected to be found in those foods. The maximum combined limits for aflatoxins B_1, B_2, G_1, and G_2 in foods are regulated, ranging from 15 µg/kg in products such as almonds, pistachios, and Brazil nuts, to 4 µg/kg in dried fruits and processed cereal products. However, because aflatoxin B_1 has relatively higher toxicity and abundance, its maximum limits are stated individually. Furthermore, since AFM_1 is a product of AFB_1 metabolism, and is a health concern, its presence in milk and dairy foods is allowed at very low concentrations ranging from 0.05 to 0.025 µg/kg in raw or heat-treated milk, and in foods for special medical purposes for infants, respectively (EC, 2006; Bahrami *et al.,* 2016).

Table 1. Tolerable daily intake of some important mycotoxins in foods.

Mycotoxin	**Tolerable daily intake (µg/kg bw)**
Ochratoxin A	0.017
Aflatoxin B_1	NA*
Aflatoxin M_1	0.001**
Patulin	0.4
Deoxynivalenol	1.0
Zearalenone	0.25
Fumonisins	2.0
Nivalenol	1.2
Sum of T-2 and TH-2 toxin	0.1

*Not available. Aflatoxin B_1 is considered the most toxic mycotoxin. Its consumption should be absent or maintained as low as reasonably achievable (ALARA). **Aflatoxin M_1 is a product of metabolism of AFB_1. Its consumption at 0.001 µg/kg (1 ng/kg) is considered as safe.

Ochratoxin A is principally produced by *P. verrucosum* and *A. ochraceus*. This mycotoxin is frequently found in processed and unprocessed cereals for adult and children consumption, as well as in fruits and fruit products, roasted and soluble coffee, wine, and spices (Bui-Klimke *et al.,* 2015). Its concentration in products for direct consumption must be lower than the allowable limits in products that will be used as ingredients in foods. For instance, the content of OTA allowed for unprocessed cereals is 5.0 µg/kg, whereas processed products are allowed to contain a maximum of 3.0 µg/kg (EC, 2006).

The presence of DON, zearalenone, and fumonisins (FB_1 and FB_2) in contaminated foods is associated with prior contamination of *Fusarium* spp. in grains. The European regulations have established maximum limits for DON, zearalenone, and the sum of FB_1 and FB_2 in cereals, and their derivative products intended for direct consumption such as pasta, bread, and breakfast cereals. A proportion of the content of the mycotoxin content in the grain is expected to be eliminated during processing. Thus, significantly higher concentrations are allowed in raw products than in foods intended for direct consumption (EC, 2006). For example, the maximum content of DON allowed in unprocessed corn is 1750 µg/kg, whereas in bread, the maximum value allowed is 750 µg/kg (EC, 2006). Similarly, the maximum level of zearalenone allowed in refined corn oil and unprocessed corn is 400 and 350 µg/kg, respectively, but in food products derived from corn such as snacks and breakfast cereals, the maximum content allowed is 50 µg/kg, and that in food for infants and young children is only 20 µg/kg (EC, 2006).

Patulin is another type of mycotoxin that is important in foods, and is therefore regulated. Patulin is mainly produced by *P. expansum*. It is found in fruits, fruit juices, and fruit nectars especially in apples, pears, and food products made from them (Adeyeye, 2019). Its maximum allowable level in these products is 50 µg/kg; but, when the products are intended for consumption by infants and young children, the maximum content permitted is five times lower (FDA, 2000; EC, 2006).

Limited information is available regarding the occurrence and levels of other dangerous fungal metabolites in foods (Kovač *et al.,* 2018). Since their presence is usually low, and that they co-occur with regulated mycotoxins, their specific regulation is presumed to be unnecessary. These compounds include diacetoxyscirpentriol, T-2 toxin, HT-2 toxin, nivalenol, beauvericin, fusarenon-X, enniatins, moniliformin, verrucol (EC, 2006; Oueslati *et al.,* 2018), alternariol monomethyl ether, alternariol, and tenuazoic acid, as well as many other compounds that are considered as emerging and masked mycotoxins, whose occurrence in foods has been found to be high under several investigations (Jajić *et al.,* 2019; Panasiuk *et al.,* 2019). Further investigation of these compounds, their toxic effects, and their co-occurrence is necessary to establish their importance as mycotoxins in foods (Alassane-Kpembi *et al.,* 2017; Spanic *et al.,* 2020) and the importance of their regulations.

Presence of mycotoxins in foods

Foods may contain one or several mycotoxins produced by different fungi simultaneously (Eskola *et al.,* 2019), according to the pathogen specificity for the crop and its processed products. For example, OTA is mainly detected in cereals, but it may also be found at high levels in beer, fruit juices, wine, coffee, spices, and dried fruits. Similarly, fumonisins and trichothecenes are regularly found in corn and food products made from them (De Girolamo *et al.,* 2016).

The presence of mycotoxins in beers is mainly derived from the growth of *Fusarium* spp. in barley, although the grains can also be colonised by *Aspergillus* spp. and *Penicillium* spp. (Mastanjević *et al.,* 2019). Most mycotoxins are thermostable and resist the operations applied during the malting and brewing process (Mastanjević et al., 2019). Thus, mycotoxins generated by *Fusarium* are frequently found in beers. These include DON, nivalenol, HT-2, T-2, zearalenone, fumonisins, and others such as deoxynivalenol-3-glucoside, fusarenon-X, and 3-acetyl deoxynivalenol (Mastanjević *et al.,* 2018). In addition, OTA, AFB_1, AFB_2, and alternariol have been detected in craft and industrially produced beers from diverse regions worldwide. Although differences in the prevailing mycotoxin depend on geographic origin, DON and zearalenone are frequently the abundant mycotoxins in beers (Peters *et al.,* 2017; Pascari *et al.,* 2018).

The geographic origin and handling of products can cause significant differences in the infestation and development of certain types of fungi. A survey conducted in Spain has reported the presence of AFB*1* and AFB*2* in pistachios from Iran, but not in pistachios from the United States, Turkey, or Spain (Ariño *et al.,* 2009). In another study conducted in Italy, samples of dried fruits, particularly raisins from Turkey, have been found to contain OTA at concentrations ten times higher than those found in the samples from other origins, and exceeded the recommended limit for consumption according to the European Commission (Fanelli *et al.,* 2017).

The presence of mycotoxins in foods and feeds can pose a major risk to human health. However, there is a lack of legislation for most of these compounds (Kovač *et al.,* 2018). For example, there is no legislation pertaining to the maximum patulin concentrations in foods in several countries. In a study conducted in Mexico, the amount of patulin in apples and food products made from them ranged between 1,500 and 30,000 µg/kg, which was higher than the 50 µg/kg allowed by the European Community, thus demonstrating the need for regulation in this respect (Hermosillo *et al.,* 2015).

The development and use of fungal resistant varieties of plants and the application of good agronomic practices contribute to diminishing the presence of fungal infestation in foods and consequently the risk of mycotoxin presence in these foods. Traditional agronomic practices involve the use of fungicides to control phytopathogen infestation. Some authors have reported a decrease in the incidence of *Fusarium* head blight, and a lower content of DON and zearalenone when prothioconazole is applied in the post-anthesis phase of wheat growth (Kharbikar *et al.,* 2015). Prosaro® applied during the flowering period in wheat has also been reported to help restrain the growth of *Fusarium*, thus suppressing the production of regulated and emerging mycotoxins and the contamination of wheat grains and malt, but not beer (Mastanjević *et al.,* 2018). There are controversial results about the effectiveness of reducing the fungal infestation in crops when they are cultivated in organic or conventional systems. In research conducted by Bernhoft *et al.* (2010) in Norway, 602 cereal products were analysed. Organic produce showed less infestation and content of DON, HT-2, and T-2 toxins than conventionally grown produce. In contrast, other researchers have not observed differences between unprocessed cereals or cereal-based products generated through organic or traditional systems in terms of the content of several mycotoxins such as OTA, AFB_1, DON, and fumonisins (Pleadin *et al.,* 2017), or the content of some type of B trichothecenes, such as DON, fusarenon-X, and nivalenol in wheat crops. Some authors have even reported higher levels of mycotoxins, such as enniatins in organically produced than conventionally produced wheat (Stanciu *et al.,* 2017). Additionally, a study comparing samples of meat from pigs raised in a conventional or alternative system has found a higher concentration of OTA in the latter, even when the content in the feed did not differ (Meucci *et al.,* 2019). Given the discrepancies in the results obtained, further investigation on this topic is necessary.

Risk assessment of mycotoxin consumption

The risk of mycotoxin intake is commonly measured by relating the frequency of food consumption to the concentrations of mycotoxins found in samples of the most consumed foods in a geographical area. This information is usually associated with the study participant data to identify the levels of toxins consumed per unit body mass (Franco *et al.,* 2019; Coppa *et al.,* 2020). Some studies have analysed the correlations between levels of mycotoxins in the urine or blood, and the self-reported food intake to calculate the risk of mycotoxin consumption (Cantú-Cornelio *et al.,* 2016; Azarikia *et al.,* 2018). Understanding the associations between the concentrations of original mycotoxins or their metabolised products in liquid human samples of different age groups and food preferences, or dietary habits contributes to better identification of vulnerable consumer groups (Mally *et al.,* 2016). From this perspective, several studies have indicated that children might frequently exceed safe consumption levels for several regulated and emerging mycotoxins in multiple regions of the world, including developed and developing countries (Ojuri *et al.,* 2018; Franco *et al.,* 2019; Gratz *et al.,* 2020; Silva *et al.,* 2020). Due to the diversity of methodological approaches, the comparison between studies is sometimes difficult. Studies conducted with the same methodology have indicated that the risk of mycotoxin consumption is usually higher in developing countries and may differ among regions and even seasons of the year (Ojuri *et al.,* 2018; Franco *et al.,* 2019). Even developed countries are not exempted from the presence of mycotoxins. Research conducted in Germany by Hickert *et al.* (2016) has analysed a sample of 96 food products such as tomato products, bakery products, sunflower seeds, fruit juices, and vegetable oils, and reported the presence of at least one mycotoxin produced by *Alternaria* in 92% of the samples. The report suggests a wide distribution of mycotoxins in foods commonly consumed in Germany.

The food consumption habits of a population can constitute another reason for differences in the intake of mycotoxins across geographical areas (Alberts *et al.,* 2016). For example, in a study conducted by Gerding *et al.* (2015), the levels of DON, OTA, and AFM_1 were analysed in the urine of people living in Bangladesh, Haiti, and Germany. Higher levels of DON were detected in people from Germany and Haiti than in Bangladesh, and metabolites residues from the ingestion of OTA were present in urine from the three populations but were higher in the people living in Bangladesh, whereas AFM_1 was found exclusively in people from Haiti and Bangladesh. Evidence of frequent and excessive exposure to regulated mycotoxins such as DON, OTA, zearalenone, their metabolised products, and also emerging mycotoxins has been reported after analysis of urine samples of 120 people from Nigeria (Šarkanj *et al.,* 2018). The evidence of the intake of DON and OTA above recommended levels has also been reported in an analysis of urine samples of 40 pregnant women from Croatia (Šarkanj *et al.,* 2013).

Evidence have indicated that aflatoxin consumption from contaminated foods may be higher in developing countries (Yard *et al.,* 2013;

Gruber-Dorninger *et al.,* 2019). In that sense, a study conducted in Mexico has reported that some frequently consumed foods such as rice, beans, peanuts, and tortillas exhibited AFB_1 levels between 15 and 250 µg/kg, levels markedly higher than the 20 µg/kg allowed according to regulations (Guzmán-de-Peña and Peña-Cabriales, 2005). This aflatoxin is metabolised and its product, AFM_1, can be found in breast milk. Elevated incidence of AFM_1 in breast milk has been reported in developing countries such as Iran, Tanzania, and Turkey. The presence of AFM_1 in breast milk has been detected in 100% of samples in Iran, and associated with consumption of bread, flour, egg, and dough (Azarikia *et al.*, 2018). In a study in Tanzania, 84% of plasma samples from 148 children presented AFM_1, which was associated with contaminated corn consumption by nursing mothers (Shirima *et al.,* 2013).

Seasonal variations in food consumption can affect the mycotoxin content in milk, as demonstrated in a study in south-eastern Turkey, where the incidence of AFM_1 ranged from 87.5% in December to 91.2% in June, with concentrations between 11.3 - 27.8 to 9.6 - 80.0 ng/L, respectively (Altun *et al.,* 2017). Since AFM_1 is recognised for its potent carcinogenic effects, many efforts have been made to analyse its content in milk. Nevertheless, the content of regulated mycotoxins other than AFM_1, as well as emerging mycotoxins in milk, has received increasing attention by researchers in recent years. Consequently, beyond OTA and AFM_1, the presence of beauvericin and enniatin B has been observed in breast milk samples from women living in Nigeria (Braun *et al.,* 2018). Furthermore, in a study of 122 mothers in Turkey, the presence of AFM_1, DON, and zearalenone has been detected at reduced levels in breast milk samples, but the concentration of OTA exceeded the maximum acceptable content of 0.5 ng/mL in 97.5% of samples. Interestingly, that report has proposed that smoking exposure may strongly contribute to increasing the risk of transferring OTA to milk (Memiş and Yalçin, 2019).

AFM_1 in animal milk, like that observed in human milk, shows differences in incidence and concentrations across regions. In a study conducted in Iran, AFM_1 has been reported to be present in 55.6% of cow milk samples at levels of approximately 21.6 ng/L, which is far below the 100 ng/L allowed in milk for human consumption according to Iranian legislation, although 30% of the samples exceeded the 50 ng/L allowed by European regulations (Hashemi, 2016). In a contrasting study, none of the 191 cow milk samples from several regions in Spain exceeded the levels of AFM_1 established by the European Union (Flores-Flores and González-Peñas, 2018). Again, the risk of mycotoxin intake may be lower in developed than in developing countries, and this difference may be related to the level of commitment of the authorities (Yard *et al.,* 2013; Matumba *et al.,* 2017).

Treatments to reduce mycotoxin concentrations in foods

The prevention of fungal growth in raw ingredients and foods for direct consumption remains the primary way to diminish the risk of mycotoxin presence in foods and feeds. The recommended procedures to reduce fungal infestation includes the application of good agronomic practices (Adeyeye, 2019; Agriopoulou *et al.,* 2020). Care must be taken during harvest, transport, and post-harvest storage to avoid mechanical damage to prevent the formation of fungal entry points into grains and subsequent toxin production.

The control of humidity and temperature during the storage of products is crucial because some of the principal mycotoxin-producer fungi can grow at water activities (a_w) higher than 0.70, and usually generate mycotoxins at a_w higher than 0.90 (Agriopoulou *et al.,* 2020). This threshold has been demonstrated for species such as *A. flavus* and *A. parasiticus* which produce aflatoxins in cereal grains, and for *A. ochraceus* and *P. verrucosum* which produce OTA in coffee, grapes, spices, and cereal grains. It has also been proven that *P. verrucosum* can grow in cereals at humidity levels between 16 and 17%, but it produces mycotoxins only when the humidity is 1% higher (Codex Alimentarius, 2002). Additionally, *A. alternata* is capable of producing mycotoxins at 0.90 a_w over the range of 10 - 35°C and produces small amounts of mycotoxin even at 6°C (Lee *et al.,* 2015).

Treatments applied to raw materials throughout the conditioning and transformation process such as cleaning, cooking, baking, frying, roasting, and extrusion aim to make them suitable for consumption. These treatments can contribute to reducing the concentrations of mycotoxins in foods. However, their effectiveness varies (Kaushik, 2015).

The distribution of mycotoxins in the fractions obtained from milling of cereals for flour production has been studied. The milling operation does not involve temperature application. Thus, it does not destroy mycotoxins, but it decreases their concentrations in the portions intended for human consumption, and increases their concentrations in the fraction intended for animal consumption. Since the outer layer of the seed is the first part colonised by fungi, it exhibits higher levels of mycotoxins. After the wheat grain is broken, the bran is separated, thus decreasing the mycotoxin content in the flour (Pinotti *et al.,* 2016). The efficiency of the reduction of the mycotoxin content during milling varies considerably. The final content of the trichothecenes T-2 and HT-2 may range from 7 to 63% of the initial amount contained in the grains (Pascale *et al.,* 2011). An increase in the content of fumonisins in the feed products of corn after the milling process has also been reported (Bordini *et al.,* 2019).

Nixtamalisation is alkaline cooking of corn grains for several minutes before making tortillas. Nixtamalisation may degrade between 90%, and nearly to 100% of the AFB_1 present, even in very highly contaminated grains (Moreno-Pedraza *et al.,* 2015). A substantial decrease in the content of aflatoxins and zearalenone (73.0 and 72.9%, respectively) has been reported in nixtamalised corn flour (Kanwal *et al.,* 2018). Other authors have reported a significant decrease in the content of FB_1 and FB_2, and their hydrolysed forms in corn, following nixtamalisation (De Girolamo *et al.,* 2016), and significantly less toxic effects on rats fed with nixtamalised products (Voss *et al.,* 2017). The results of those reports are associated with the vulnerability of the molecule to thermal-alkaline treatments. Unfortunately, this process has some technological and ecological disadvantages, and thus other technologies are favoured in the industry to obtain dough to make tortillas.

Roasting, cooking, baking, frying, and extrusion techniques increase the temperature of the food materials treated, and their efficacy in reducing the content of toxins in food varies. The time and temperature conditions of the treatments, the food matrices, on which they are applied, and the thermostability of the mycotoxins tested are the principal causes of the differences in reduction (Kaushik, 2015). The members of the aflatoxin family and OTA are very thermostable (Gbashi *et al.,* 2019). Therefore, they are frequently used as indicators of the efficacy of thermal treatment. Roasting pistachios at 120°C for 20 min, according to commercial practice, does not reduce AFB_1 concentrations in naturally contaminated samples (Ariño *et al.,* 2009). To achieve a decrease above 93%, the addition of lemon juice and citric acid, and roasting at 120°C for 60 min are necessary. However, this treatment alters the physical properties of pistachios (Rastegar *et al.,* 2017). The effect of roasting coffee beans decreases the content of aflatoxins from 42 to 56%, and the content of OTA from 13 to 93%. Extrusion can generate significant reductions in the content of OTA, aflatoxins, zearalenone, and fumonisins in several products (Karlovski *et al.,* 2016). The content of FB_1 in corn can be diminished by as much as 85%, thus substantially decreasing its toxicity (Voss *et al.,* 2017). However, the results depend on several conditions such as the food treated, the type of extruder, the barrel temperature, the moisture content of the raw material, and the use of additives such as sugar, salt, or ammonium bicarbonate (Karlovski *et al.,* 2016; Voss *et al.,* 2017). The effects of cooking on the reduction of mycotoxin concentration are attributable to the application of high temperatures, but a primary influence might be partial solubilisation in water (Kaushik, 2015). Cooking above the boiling point for nine minutes can decrease AFB_1 concentrations by 87.5%, and OTA concentrations by 86.6% in rice (Hussain and Luttfullah, 2009). Frying also can cause a significant reduction in fumonisin content in corn dough, but not in more stable mycotoxins such as DON (Samar *et al.,* 2007). Baking has shown controversial results, which in some cases are attributable to methodological approaches (Stadler *et al.,* 2019). Some authors have reported that baking during the bread-making process does not reduce the concentration of OTA (Vidal *et al.,* 2014). However, most experimental results have shown a decrease in DON in the range from 0 to 25% under realistic baking conditions, in products such as crackers, biscuits, and breads (Generotti *et al.,* 2017; Stadler *et al.,* 2019). Baking has a high probability of not only degrading, but also masking products of DON (Stadler *et al.,* 2019). A baking temperature of 200°C for eight minutes in the process of making biscuits from wheat flour contaminated with DON at 1,500 μg/kg has been found to result in a reduction of 68% of this compound, and reductions of 75 and 80% of the initial content of deoxynivalenol-3-glucoside and culmorin, respectively, thus yielding an organoleptically acceptable product. However, maintaining a pH of 8.0 was important for the results obtained in this experiment (Generotti *et al.,* 2017). Since extrusion and roasting involve the highest time-temperature treatments, their application results in the lowest final mycotoxin concentrations (Kaushik, 2015). In the operations involving elevated temperatures to generate thermally-formed compounds as a result of mycotoxin degradation (Stadler *et al.,* 2019), many of these compounds have not yet been identified, and their lack of toxicity has not been demonstrated. Hence further investigation remains necessary (Stadler *et al.,* 2019).

The application of radiation to foods has been a matter of debate. Its use under controlled conditions for the treatment of many foods such as meat, eggs, spices, fruits, and vegetables is authorised by regulating organisations such as the FDA. Some authors have demonstrated that the application of radiation doses of 2.5 kiloGray (kGy) or higher decreases the concentration of OTA from 90 to 100% when it is in aqueous solutions (Calado *et al.,* 2018). Furthermore, a reduction of 88% of OTA has been reported with a dose of 10 kGy applied to raisins (Kanapitsas *et al.,* 2016). Moreover, the AFB_1 production from *A. flavus* has been diminished up to 95% in irradiated peanuts (Barberis *et al.,* 2012). The concentration of zearalenone has been found to decrease up to 83% in fruit juices when a dose of 10.0 kGy was applied, although the sensory characteristics, and the total phenolic and flavonoid content were affected (Kalagatur *et al.,* 2018). The efficacy of the application of radiation on mycotoxin content depends on the food matrix, dose used, and mycotoxins and fungal species intended to be eliminated (Schmidt *et al.,* 2018). Gamma-radiation treatments at 2.5 kGy or greater show high efficacy in OTA degradation in aqueous systems, but only 24% of this compound is eliminated when an amount of 30 kGy is applied in food matrices such as grape juice, wine, and wheat flour. However, the maximum dosage recommended for use in most food products is 10.0 kGy. Applying gamma radiation to detoxify food products from OTA (Calado *et al.,* 2018), AFB_1, or DON is not recommended because doses higher than the maximum recommended levels are required (Hooshmand and Klopfenstein, 1995). Therefore, this technique should be combined with others to increase its efficiency. Furthermore, studies are lacking regarding the fate of mycotoxins and their products after radiation treatment in foods as well as the sensory characteristics of the irradiated products (Schmidt *et al.,* 2018).

The use of non-ionising irradiation in foods has yielded interesting results regarding the reduction of microbial contamination and mycotoxin degradation. The mycotoxin content in several raw materials and processed foods is significantly reduced by treatments such as ultraviolet light, microwaves, and pulsed light. However, in some cases, negative effects on the quality of food or the generation of compounds with equal toxicity have been reported. Hence, more research in this developing area remains necessary (Schmidt *et al.,* 2018).

The use of ozone gas to degrade mycotoxins has been tested and has yielded different results. The application of ozone can significantly decrease the concentrations of DON and DON-3-Glc in wheat (Agriopoulou *et al.,* 2020). The effects of ozone on mycotoxins differ, as observed in a study in which DON, OTA, and zearalenone present in naturally contaminated ground corn were degraded at up to 42.8, 70.3, and 68.1%, respectively, after application of different ozone concentrations for 120 or 180 minutes (Krstović *et al.,* 2020). The effect of ozone in significantly reducing the concentration of OTA in broiler meat has also been confirmed by immersion in ozonised water (Taher and Abdul-Shaheed, 2018).

The capacity of binding agents such as montmorillonite and bentonite to adsorb mycotoxins present in foods and feeds has been demonstrated. Hence, the use of these additives to detoxify contaminated foods has become increasingly frequent (Alberts *et al.,* 2016). Food additives can diminish the activity of mycotoxins by modifying their chemical structures (Kaushik, 2015). Some studies have demonstrated that common food additive chemicals such as lactic, acetic, and citric acids, as well as sodium chloride can decrease the concentrations of aflatoxins up to 98% in peanuts (Abuagela *et al.,* 2019). The immersion of figs in a 0.5% citric acid or a 0.3% L-cysteine solution significantly decreases the presence of AFB_1, but not OTA or other mycotoxins produced by *Alternaria* (Petrić *et al.,* 2018). The 50% decrease in DON, FB_1, FB_2, and other trichothecenes by using lactic and citric acids at 5% solutions has also been reported in feeds, but the resulting compounds and their toxicity have not been evaluated (Humer *et al.,* 2016).

The treatment of raw food with probiotic microorganisms has shown promising results. Several investigations have focused on inactivating mycotoxins by applying lactic acid bacteria (LAB) as well as their metabolites and/or enzymes. Inoculated LAB have been demonstrated to prevent the growth of *Fusarium* on barley during the brewing process (Peyer *et al.,* 2017). The use of a mixture of yeasts (*Kluyveromyces lactis* and *Saccharomyces cerevisiae*) and LAB (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) inoculated in baby foods made from wheat has been shown to reduce the concentration of aflatoxins B_1, B_2, G_1, and G_2 by as much as 93% (Hamad *et al.,* 2018). The application of yeasts has been demonstrated to reduce the concentrations of mycotoxins in products by binding DON, OTA, zearalenone, and FB_1 on cell membranes (Agriopoulou *et al.,* 2020). *Saccharomyces cerevisiae* can reduce OTA by 70 to 90% during the fermentation of wine (Csutorás *et al.,* 2013), and approximately 59% throughout the bread-making process (Mozaffary *et al.,* 2019). Investigations using no probiotic yeasts have also exhibited promising results in which decreasing the growth of several OTA-producing fungi by 50 to 70%, whereas the release of OTA has been found to be almost completely repressed in the presence of antagonistic yeast strains (Farbo *et al.,* 2018). Another investigation has reported the detoxification of corn oil with an enzyme produced by *Pichia pastoris*, thus decreasing zearalenone from 1257.3 to 13.0 µg/kg, and forming degraded products with substantially diminished toxic effects (Chang *et al.,* 2020).

Detoxification of products can be achieved by decomposing mycotoxins into simpler structures or decreasing their concentration by removal (Rastegar *et al.,* 2017). Using microorganisms for detoxification of foods and feeds has shown promising results (Ahad *et al.,* 2017; Gao *et al.,* 2018). The metabolism of mycotoxins by microorganisms contributes to the formation of simpler compounds. However, much work remains to be done because some compounds have not yet been identified and may possess toxic properties (Ahad *et al.,* 2017). The use of microorganisms may also contribute to diminishing the concentrations of mycotoxins by binding them to cell membranes and forming conjugates (Lili *et al.,* 2018). However, when consumed, those chemical compounds may be hydrolysed, thereby releasing mycotoxins in their initial, hazardous configurations. In both cases, more research on the proportion of the formation of new compounds and the amount of primary mycotoxins liberated must be conducted.

Perspectives

Mycotoxins are produced by fungi as secondary metabolites, mainly under stress conditions. Knowledge of the agronomic practices and postharvest handling needed to reduce the incidence of fungal growth and mycotoxin production in foods is important and a focus of several research groups worldwide. Moreover, environmental conditions associated with climate change, such as alterations in temperature, precipitation, and atmospheric composition can affect the patterns of distribution and the incidence of pathogens and their metabolism. Therefore, an increase in the production of mycotoxins is possible (Jajić *et al.,* 2019), thus making this issue a global challenge (Kovač *et al.,* 2020).

The increase in epidemiological studies on the consumption of the main mycotoxins and their effects on human health remains relevant. The sanitary regulations imposed by governments from diverse countries and economic regions differ (Eskola *et al.,* 2019; Vaz *et al.,* 2020), and sometimes can be used to facilitate or to obstruct trading between countries, thus causing food with elevated levels of mycotoxins to be concentrated in the poorest areas of the planet (Matumba *et al.,* 2017). Maximum limits for emerging and masking mycotoxins as well as regulated ones must be established according to the health implications for the people who will consume them, because exposure could differ according to diverse food consumption habits (Matumba *et al.,* 2017).

The characterisation of the distribution of mycotoxins in grain structure remains a fascinating issue for many researchers. The economic and health effects of concentrations of these compounds in feeds are vast (Pinotti *et al.,* 2016). A large amount of feed worldwide might be exceeding the permissible limits, thus resulting in financial losses to producers (Eskola *et al.,* 2019). The consumption of animals given feed containing high levels of mycotoxins and their by-products might translate to an excessive intake of residual mycotoxins by humans. Therefore, determining the distribution of mycotoxins in grains is crucial to guide treatments to reduce the contamination of the resulting fractions in the milling process.

Research to reduce the levels of mycotoxins in food from the field has included some proposals to incorporate microorganisms in treatments. This technique has been applied at *in vitro* studies in which non-toxic DON derivatives were placed in contact with intestinal tissue cells (He *et al.,* 2015; Pierron *et al.,* 2016). Furthermore, *in vivo* studies in which livestock were fed both toxins and bacteria capable of transforming DON have yielded favourable results (Grenier *et al.,* 2013), or have demonstrated less toxicity than that of the primary mycotoxin (He *et al.,* 2015); however, in some cases, compounds with the same or unknown toxicity are produced. Accordingly, regulations on the permissible limits of these compounds in foods must be established. The health authorities are expected to issue regulations in this regard in the coming years (Pinotti *et al.,* 2016).

Consumers of organic foods, hand-made food products, or commodities with high antioxidant content expect to obtain health benefits from ingesting them. However, information about whether the organic production process yields products with lower mycotoxin concentrations than conventionally produced products is uncertain. Therefore, the regulation and supervision of organically produced foods is another relevant issue to be investigated in the future.

Conclusions

Mycotoxin contamination has been extensively studied, and many efforts have been made to regulate mycotoxin content in foods and feeds. However, knowledge is available for only several mycotoxins. Research on the occurrence, toxic effects, and exposure of emerging and masked mycotoxins is a challenge to tackle.

Mycotoxin contamination exists in the entire food production chain. Fungal infestation in foods is highly dependent on agricultural management and environmental conditions during harvest and post-harvest, thereby determining the appearance of mycotoxins in harvested commodities. Scientific evidence indicate that climatic change has altered the occurrence patterns of some fungal species and their mycotoxin production in some regions of the world, thereby constituting a global concern. Thus, more efficient control of mycotoxin production through enhancing agronomy and post-harvest practices should be pursued.

Research on physical and chemical treatments to detoxify foods and feeds have led to substantial achievements; but, complete detoxification is not yet possible. More advances in this topic are expected in the near future.

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