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Mycotoxins: The threat to food safety

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Abstract

Food safety is the practices that ensure food does not cause harm whereas food safety hazard is any factor found in food that has potential threat or causes harm to the consumer, by causing injury or illness. Mycotoxins are significant food safety hazards representing a major threat to human and animal health. They are naturally occurring chemical hazards that can produce by certain genera of fungi, *Asperigllus*, *Fusarium*, and *Penicillium* as secondary metabolites. The major types of mycotoxins that have great effects on food safety and human health are aflatoxins, ochratoxins, patulin, fumonisins, zearalenone, and trichothecenes. Mycotoxins are very stable molecules and their occurrence in food can be pre- or post-harvest stages. Prevention is an important strategy to control mycotoxins and should be achieved in pre-harvest and during storage stages, in both raw materials and processed food. Also, there are many methods for decontamination or detoxification which applied to food or feed contaminated with mycotoxins, without affecting the quality, the properties, or the safety of the food or feed. Continuous programs should be established to monitor the mycotoxins levels in food products during storage, distribution, and marketing to prevent any adverse effects on food safety and rather consumer health. Different methods were used for mycotoxins determination in food and feed such as TLC, HPLC, HPLC-MS, HPLC-MS/MS, and GC-MS. This review highlights chemistry, sources, occurrence, stability, prevention and control strategies, detection methods, and legislation of the most important mycotoxins with special reference to the international and Egyptian standards.

Keywords: Mycotoxins, food safety, chemical hazards, decontamination, legislation

1. Introduction

Food safety is an important issue that affects people's health all over the world. Many countries are increasingly dependent on the availability of their food sources and their safety [1]. The world produces enough food to feed everyone living on earth [2]. However, each year, food industries lose about 25% of their food productions due to food contamination by pathogens [3]. Food can become contaminated at any point during harvesting, processing, storage, distribution, transportation, and preparation. The definition of food safety hazard is any factor found in food that has potential threat or causes harm to the consumer, by causing injury or illness. Food safety hazards can be classified into physical hazards such as a piece of metals and stone; biological hazards like microorganisms (pathogenic bacteria and fungi), viruses and parasites; and chemical hazards, such as plant toxins, algal toxins, mycotoxins, fish toxins, biogenic amines, heavy metals, pesticides and antibiotics [4,5].

Mycotoxins are one of the major food safety hazards. Contamination with mycotoxins has been assessed as 72% of the worldwide food crops [6]. Mycotoxins problem is important worldwide due to their impact on human and animal health along with economic implications [7]. The foodborne disease burden epidemiology reference group (FERG) has been commissioned by the world health organization (WHO) to carry out the regular evaluations of some toxins such as aflatoxin, as the confession of the worldwide public health significance of foodborne illness and to encourage the development and growth of the world economy [8]. It is important to know the relevant data on the major weather conditions in the agricultural zones of the cultivated crops, to understand the mechanisms that can be applied to control mycotoxins. In developing countries, the fungal growth and mycotoxin production can be enhanced as a result of high moisture content and

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high temperatures, unseasonal rains, monsoons during harvest [9].

2. Mycotoxins

The mycotoxin term is derived from the Greek word (mycosis) which means fungus and the Latin word (Toxicum) which means poison. It is designated the naturally occurring toxic chemical substances produced by certain species of fungi, as secondary metabolites. It has been estimated that there are between 100,000 and 1,500,000 fungal species, producing between 200,000 to 3,000,000 secondary metabolites. At least there are approximately 70000 recognized fungi species and 500 known mycotoxins [10]. Mycotoxins are defined as low-molecular-weight compounds produced by filamentous fungal genera like *Aspergillu*s, *Fusarium*, *Penicillium*, *Alternaria*, and *Claviceps*.

In 1962 the expression mycotoxin has first used the aftermath of uncommon mortality of about 100,000 turkey poult near London. The relation between turkey X disease and the peanut contamination with Aspergillus flavus metabolites warned the researchers from the other fungal metabolites which might be deadly [11]. After that, the list of mycotoxin was extended to include formerly known fungal toxins and the other compounds that had been initially isolated as an antimicrobial agent e.g., patulin, and the novel toxic secondary fungal metabolites e.g., ochratoxin A. Around three to four hundred compounds are recently categorized as mycotoxins; however, nearly twelve groups that threaten human and animal health receive great attention [12]. Although all mycotoxins are fungal products, not all toxic agents that are excreted from fungi are classified as mycotoxins. On the other hand, there are fungal metabolites that have toxic activity against bacteria (for example penicillin) are called antibiotics, while those that show toxic activities against plants are called phytotoxins [13].

Mycotoxins are difficult to classify where classification schemes mainly depend on the specialty of the person who classifies. Clinicians classify mycotoxins according to the affected organ (hepatotoxins, nephrotoxins, etc.). Cell biologists categorize mycotoxins as teratogens, mutagens, carcinogens, and allergens. Organic chemists classify them according to the chemical structures (coumarins, lactones, etc); bio-chemists according to their biosynthetic origins (amino acid-derived, polyketides, etc.); physicians according to the illnesses they cause and mycologists according to the producing fungi (e.g., *Aspergillus* toxins, *Penicillium* toxins). The same compound might have different names. Aflatoxin, for example, is sometimes called hepatotoxic, mutagenic, carcinogenic, difuran-containing, polyketide-derived Aspergillus-toxin. The Fusarium metabolite Zearalenone has a potent estrogenic hormonal activity [14]. From the health and trade point of view, the major mycotoxins that receiving attention are aflatoxins, ochratoxins, fumonisins, patulin, deoxynivalenol, zearalenone, alternariol, and trichothecenes [15].

3. Factors effect on fungal growth and mycotoxins production

The favoring conditions for fungal growth and mycotoxin production relate mainly to pre-harvest treatments and poor hygienic practices during transportation, processing, incorrect storage like high temperature, moisture content, and heavy rains [14]. Specialists have found a variety of factors that favor the production of mycotoxins. Those are grouped as physical, chemical, and biological factors (Figure 1). Physical factors include environmental conditions like temperature, relative humidity, and insect infestation. While chemical factors include the use of fungicides, pesticides, or fertilizers, as well as biological factors, depend upon the interactions between toxigenic fungi and substrate. Some plant species are more susceptible to fungal colonization while environmental conditions may increase the vulnerability of others are more resistant [16]. Also, thus factors can be divided to either extrinsic, intrinsic, processing, or implicit which including moisture content, water activity, temperature, climate, oxygen level, type of substrate, type of plant, and nutrient composition; drying, blending, preservatives addition, handling of grains; insect interactions, fungal strain and microbiological ecosystem [17]. In this review, the factors that affect the occurrence of fungal growth and mycotoxins production will be categorizing by different classifications according to pre-harvest and post-harvest conditions.

3.1. Pre-harvest factors

Pre-harvest conditions like the type of soil, soil condition, drought, Genotypes (breeding plants resistant to fungal infection), plant density, level of fertilization, and insect activities are the most important in determining the probability of pre-harvest contamination [18]. Soil is a natural factor

that exerts an effective influence in the fungal infection occurrence. Crops grown in different soil types may have a significant effect on fungal growth and levels of mycotoxin contamination. For example, peanuts grown in sandy soils promote rapid fungal growth, especially under dry conditions, while less contamination of peanuts in heavier soils due to their high holding capacity of water which helps the plant to prevent drought stress [19].

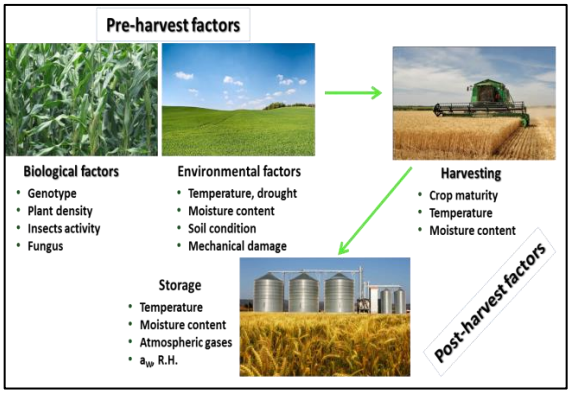

Fig. 1. Principal factors influencing fungal growth and mycotoxin production.

Invasion of cereals by insects decreases the grade, quality, and market value of the agriculture products which in most instances are considered unsafe for human and animal consumption. Pest infestation damage is a suitable condition for the growth of mycotoxigenic fungi and mycotoxins contamination. Avantaggio et al. [20] reported that damage of maize due to insect invasion is a good indicator of Fusarium toxins contamination. Also, insects carry spores of mycotoxigenic fungi from plant surfaces to the interior layers of grains or create infections to other plants through their feeding habits [21].

Harvest is the first step in the chain of production where water activity (a_W (food) = relative humidity/100) and moisture content become the most important parameters in the management and protection of the crops from fungal infection and subsequently mycotoxins contamination. Grains should be harvested in the ideal time after the period of dry weather when it is at safe moisture content. Another important control point at harvest will be visual monitoring of the grains for symptoms of disease, color sorting, density segregation, mechanical separation of diseased batches from healthy grain [22]. Early harvesting of crops reduces fungal infection in the field and consequent mycotoxins contamination of harvested products and delayed harvest can result in increased contamination with mycotoxins. Kaaya et al. [23] found that aflatoxin levels increased 4 times by delaying the harvesting for 3 weeks and more than 7 times for 4 weeks. Whereas, products should be dried to safe levels at harvesting in a suitable time to stop fungal growth.

3.2. Post-harvest factors

The post-harvest steps are the stages following harvest which lead to primary processing like milling. The post-harvest treatments including drying, storage, transportation, and processing steps considered critical control points in fungal growth and mycotoxins contamination prevention strategies. Poor post-harvest management can lead to rapid losses in the nutritional quality of food products [22]. Rapid drying of crops to minimal moisture level is critical as it creates unfavorable conditions for fungal growth and insect infestation and helps to keep products longer without any damage [24]. Aflatoxins contamination can increase 10 folds within 3 days by storing maize at high moisture contents. The general recommendation to prevent fungal growth and mycotoxin contamination is that harvested crops should be dried as soon as possible to save the moisture level of 10 – 13% which can be achieved through simple sun-drying [25].

Storage is a critical stage where fungal infection, insect infestation, and mycotoxins accumulation may occur. Mycotoxins contamination of feeds and food may result from unsuitable storage conditions or handling of food products. The growth of toxigenic fungi on grains is influenced by the storage conditions like water activity (a_W), the temperature of the substrate, aeration, microbial interaction, insects, and rodent activity [26]. Control of moisture content is the main point to avoid mycotoxins accumulation in stored grains. Temperature 10-40 ◦C, pH 4-8, and aW at levels above 0.70 are the conditions in which fungi usually develop [27]. *Aspergillus flavus* require a_W more than 0.85 and temperature > 10ºC for germination and growth, A. ochraceus can grow at 10ºC with aW from 0.85 to 0.87. While patulin production by *Penicillium expansum* can occur in a wide range of temperatures between 0 to 24ºC. Moisture content requirements vary widely among Penicillium species, some Penicillium spp. can grow on substrates with no water content [28].

4. Economic impacts of mycotoxins

Economic losses due to mycotoxins are varied and can lead to a reduction of qualified foods for humans and animals, reduction in the production of the animal as a result of feed rejection or diseases, high medical cost to treat toxicosis, high cost to search for alternative foods, improving the detection and quantification methods and developing strategies to decrease mycotoxin exposure [29]. From the international trade point of view, mycotoxins contamination inflicts huge economic loads. It decreases the price cost for crops and can cause losses of large amounts of food. In the USA, losses from mycotoxins – in the hundreds of millions of US dollars annually – are usually associated with these market costs in addition to human health effects [30]. Robens and Cardwell [31] reported that aflatoxin contamination has affected millions of hectares of peanuts and maize crops and loss ranging from $0.5 million to more than $1.5 billion for the USA as a result of Aflatoxins contaminated corn and peanuts, in addition to Fumonisin B contaminated wheat. Vardon et al. [32] calculated potential losses annually of three mycotoxins, fumonisin, aflatoxin, and deoxynivalenol to range between $418 million to $1.66 billion from wheat, corn, and peanuts produced in the USA. Additionally, they reported that the cost of livestock losses could include another $466 million per year. Loss in this broadly consumed cereal due to mycotoxins may have painful economic impacts worldwide not only for the producers.

The economic impact of mycotoxins contamination in Africa can be measured through the decrease of food availability, specifically, among the poor areas, regulatory rejection of exported goods, a decrease of the market value of contaminated products in domestic markets, reduced marketability of crops which considered a clear food security threat, as well as increased livestock, human diseases, and mortality. Moreover, this impact will extend to increase the cost of research and regulatory activities that are aimed at reducing the potential risks of mycotoxins on human and animal health [33]. EU regulation of mycotoxins was expected to reduce African export of nuts, cereals, oilseeds, and dried fruits by 64%, reportedly costing 670 million US$ yearly [34]. The International Institute of Tropical Agriculture IITA [35] reported the annual economic losses of 1.2 billion US$ on a global scale due to aflatoxins contamination, and 38% of this loss (450 million US$) is recorded by African countries.

5. The major types of mycotoxins

5.1. Aflatoxins

Aflatoxins are a group of naturally occurring toxic chemical hazards produced as secondary metabolites of certain species of fungi. Aflatoxins are highly toxic compounds which can cause acute and chronic toxicity in human and animal [36]. The aflatoxins were first isolated and identified after the disaster of more than 100,000 turkey poults died in the USA and England after consumption of a mold-contaminated peanut meal which identified as a causative agent in Turkey X disease that causes liver necrosis [37]. There are more than 20 known similar aflatoxin compounds, but only four compounds are found naturally in foods. These are aflatoxins B1, B2, G1, and G2 (Figure 2). These abbreviations according to their fluorescence color they acquired under the UV lamp (blue or green) and relative distance through the TLC plate [38]. While aflatoxin M1 and M2 are the metabolic products of aflatoxins B1 and B2 (hydroxylated metabolites of B1 and B2) isolated from animal milk-fed on grains contaminated with aflatoxins and they are potentially important contaminants source in dairy products [39].

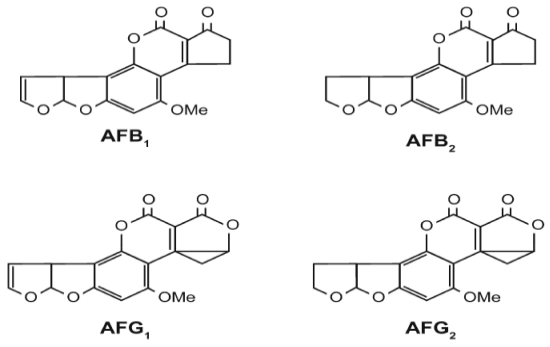

Fig. 2. Major aflatoxins

Aflatoxins are difuranocoumarin derivatives structurally related to coumarin which is produced by a polyketide pathway by many strains of *Aspergillus flavus* and *A. parasiticus*; in particular, *A. flavus* is a common contaminant in agriculture. *A. bombycis*, *A. ochraceoroseus*, *A. nomius*, and *A. pseudotamari* are also aflatoxin-producing species, but they are encountered less frequently [40, 41]. Aflatoxins are highly lipid-soluble compounds and are rapidly absorbed from the site of exposure through the gastrointestinal tract or respiratory tract into the bloodstream [42]. Aflatoxins get into humans and animals by direct consumption of aflatoxins-

contaminated food or ingestion of aflatoxin through milk, milk products (cheese and powdered milk), or through animal tissues mainly as aflatoxin M1. The second route by inhalation of aflatoxins in dust particles of contaminated food in factories and industries [43].

5.1.1. Aflatoxins occurrence in food

Aflatoxins are found in various food products include cereals like wheat, barley, maize, rice, sorghum, and millet; oilseeds such as soybean, cotton, groundnut, and sunflower; spices, spices like black pepper, coriander, ginger, turmeric, and chilies; and tree nuts such as almonds, pistachio, walnuts, and coconut. Figs and dried fruits are also known to be high-risk food for aflatoxins-contamination [44, 45]. Fungal infection and aflatoxins contaminated grains and cereals can occur at various steps pre-harvesting, during harvesting, transport, or storage [46]. Wheat or barley contamination with Aflatoxins is commonly happening as a result of unsuitable storage conditions. Milk and milk products like cheese and powdered milk through animal consumption of aflatoxins-contaminated feed. In milk, aflatoxin M1 is generally at 1–6% of total aflatoxins content in the feed-stuff [47]. Also, aflatoxins get into humans by consumption of aflatoxin-contaminated foods such as meat, meat products, and eggs [14, 48].

5.1.2. Aflatoxins Health effects and outbreaks

It is important to recognize that, just food becomes contaminated with aflatoxins, these toxins are very stable, and the conventional food processing treatment will be ineffective [10]. At high exposure levels, aflatoxins can cause acute toxicity and potential death in humans, mammals, birds, and fish. The liver is the principal target organ affected, but high levels of aflatoxins have also been found in the kidneys, lungs, hearts, and brains, and individuals dying due to acute aflatoxicosis. Acute cirrhosis and necrosis of the liver are typical along with edema and hemorrhaging [49]. From a food safety point of view, chronic toxicity is probably more important, especially in developed countries. Aflatoxin B1 is documented as a hepatic carcinogen and mutagen in many animals. It is listed as a Group I carcinogen by the International Agency for Research on Cancer, especially in the cause of human primary hepatocellular carcinoma. At low levels over a long period of exposure has been embroiled in primary jaundice, liver cancer and chronic hepatitis, cirrhosis [50].

The notable outbreak occurred in India in 1974 when almost 400 people become ill with Jaundice and fever after eating contaminated maize with aflatoxin (between 0.25 and 25 mg kg^{-1}) and 106 deaths were recorded [51]. Another outbreak was recorded in northwest Indian in 1974 due to aflatoxin in both humans and dogs [52]. Two major outbreaks at least have also occurred in Kenya in1981 [53]. Later, in Kenya in 2004 when 500 cases and 200 deaths were demonstrated due to aflatoxicosis outbreaks by consuming contaminated maize with molds [54]. In European countries including Croatia, Serbia, and Romania, in 2013 recorded the nationwide contamination of milk with aflatoxin M1 [55]. It is impossible to record the incidence of chronic aflatoxicosis in humans due to the difficulty to recognize the symptoms.

5.1.3. Stability of aflatoxins in food

Aflatoxins are relatively heated stable compounds and not completely degraded when treated with high temperature like boiling, autoclaving, pasteurization, sterilization, spray drying, and other processing using methods used to food preparation [56]. While, heating or cooking processes cannot be destroyed aflatoxins, the heat stability of aflatoxin is affected by some factors; such as pH and moisture content. Several studies reported that roasting is a good method for reducing the levels of aflatoxin in certain commodities, i.e., microwave roasted peanuts, oil, and dry roasted peanuts, corn, and coffee. In this concern, roasting green coffee at 180ºC/10 min caused only a 50% reduction in aflatoxin B1 level. Also, Roasting pistachio nuts at 90, 120, and 150ºC for 30, 60 and 120 min, respectively were found to reduce aflatoxin levels by17-63% [57]. The decrease in aflatoxin content depends upon the combination between time and temperature. The stability of aflatoxin M1 in cheese and yogurt has not been affected by fermentation processes [58]. Both pasteurization and boiling processes did not affect the level of aflatoxin M1 in bovine milk [59]. Aflatoxins can be destroyed by acid and alkaline hydrolysis and by the activity of oxidative agents [60].

5.1.4. Aflatoxins prevention and control strategies

The ability of aflatoxigenic fungi to grow on a wide range of food products and the stability of

aflatoxin in food lead to use the appropriate point of control by preventing the crops contamination in the field and during the storage stage or removal of contaminated food materials from the chain of food supply.

Pre-harvest control of aflatoxin is carrying out through the general Good Agricultural Practice (GAP) which includes soil preparation (to decrease or prevent the contamination of fungi producing aflatoxins), removal of crop wastes, fertilizer system application, crop rotation, and breeding crops resistant to fungi and insects infection, control of fungal infection and insect pests using fungicides and pesticides, irrigation in suitable time to prevent drought stress, harvesting the crops in suitable moisture content and suitable maturity stage [61, 62]. The use of biological control agents e.g., Bacillus subtilis, *Pseudomonas* spp., *Burkholderia* spp., *Ralstonia* spp., and *Lactobacillus* spp. are effective at management and control of aflatoxins contamination [63, 64]. Several strains of *P. solanacearum* and *B. subtilis* isolated from the non-rhizosphere of maize soil have the ability to aflatoxin elimination [65]. Biological control of aflatoxin production in crops has been approved in the USA by Environmental Protection Agency and two commercial products (AF36® and Afla-guard®) based on aflatoxigenic *A. flavus* strains are used for aflatoxin prevention in corn, peanuts, and cottonseed [66].

In post-harvest, the most effective and important control measures are handling and storage of crops by controlling the water activity and moisture content. After harvest, crops should be dried to a safe moisture level to prevent fungal growth and production of aflatoxins during storage. The safe moisture content varies between crops, it is about 14% at 20ºC in maize, but it is 7% in groundnut [25]. The moisture content must be controlled during the transportation and storage stages.

Decontamination of aflatoxins can be occurred by physical removal of contaminated materials can be effective in reducing the levels of aflatoxins in contaminated commodities, such as color sorting (remove infected peanuts), density separation, mechanical segregation, and removal of fines from nut and grain shipments also can be effective taken measures. Chemical decontamination techniques have been reported, especially in animal feed materials, but most of these investigated methods may produce toxic by-products. The Ammoniation process has been using for aflatoxin removal from the feed in the USA [67]. Ozone was used to decontaminate aflatoxins. Alencar et al. [68] noted a decrease of 25 % and 30 % of aflatoxin B1 and total aflatoxins when peanuts were exposed to 21 mg L-1 of ozone. McKenzie et al. [69] reported that ozone caused degradation of AFB1 and G1 in aqueous model systems. A 92% reduction (degradation) in aflatoxin in ozonized contaminated corn has been recorded by Prudent and King [70].

5.1.5. Aflatoxins testing

The detection and determination of aflatoxin in food and feed is a very important step for food safety ensuring. Aflatoxins are usually identified and detected according to their absorption (excitation and emission spectra). Aflatoxins B1 and B2 showed blue fluorescence at 425 nm, whereas aflatoxin G1 and G2 exhibit green fluorescence at 540 nm under UV lamps. Also, thin layer chromatography (TLC) is one of the oldest techniques used for aflatoxin detection [71]. While enzyme-linked immune-sorbent assay (ELISA), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectroscopy (LC/MS) are the most frequent methods used for aflatoxins detection and quantification [72].

5.1.6. Aflatoxins legislation

For human and animal health protection, more than 100 countries around the world have established maximum permissible levels or recommended limits for aflatoxins in food [73]. The EU set limits for total aflatoxins (B1, B2, G1, and G2) and aflatoxin B1in cereals, nuts, spices, and dried fruits. These limits are varied according to the food commodity, and ranged from 4-15 μg kg^{-1} for total aflatoxins and from 2-8 μg kg-1 for aflatoxin B1. Also, the limit of aflatoxin M1 in milk and milk products is set as 0.05 μg kg^{-1}. Recently, limits of 0.1 μg kg^{-1} for Aflatoxin B1 and 0.025 μg kg^{-1} for aflatoxin M1 have been sets for infant food [74]. USA food safety regulations set a limit of 20 μg kg^{-1} for total aflatoxins in food products and a limit of 0.50 μg kg^{-1} in milk. Both Canada and Australia set limits of 15 μg kg^{-1} for total aflatoxins in nuts. Egyptian standard set limits for total aflatoxins ranged from 4-15 μg kg^{-1} and 2-12 μg kg^{-1} for aflatoxin B1in cereals, nuts, and dried fruits. Aflatoxin B1 limit in processed cereal-based food and baby foods for infants and young children is set as 0.01 μg kg^{-1}. Whereas, aflatoxin M1 limit in raw milk and heat-treated milk and milk-based products is

set as 0.05 μg kg^{-1} and 0.025 μg kg^{-1} for infant milk, dietary foods for special medical purposes intended specifically for infants [75].

5.2. Ochratoxins

Ochratoxins are a small group of mycotoxins produced by certain species of the genera Aspergillus and Penicillium [76]. They capable of infecting the crops in both pre-and post-harvest stages lead to contaminate a wide range of feed and food products. There are three groups of ochratoxins, ochratoxin A, ochratoxin B, and ochratoxin C, which are produced mainly by *A. ochraceus*, *A. niger*, *A. carbonarius*, and *P. verrucosum*. The ochratoxins are pentaketides made up of dihydro-isocoumarin linked to b-phenylalanine. Among ochratoxins, Ochratoxin A (OTA) is considered the most toxic ochratoxin and most abundant found naturally in foods, while ochratoxin B and C are rarely found in food and much less toxic [77]. Ochratoxin A, 7-(L-β phenylalanyl carbonyl)-carboxyl-5-chloro-8-hydroxy-3,4-dihydro-3R-methyl isocoumarin (Figure 3) is a low molecular weight fungal secondary metabolite [78]. Penicillium and Aspergillus are the major OTA producers, Penicillium spp. like, P. verrucosum, *P. nordicum, P. olson, P. brevicompactum, P. crustose, P. chrysogenum*, and *P. oxalicum* have also been reported to produce OTA [79]. Also, *Aspergillus* spp. (A. *ochraceus, A. steynii, A. carbonarius, A. alliaceus* and *A. westerdijkiae*) are the major OTA producers in feed and food [80].

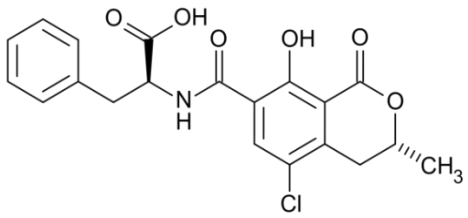

Fig. 3. Ochratoxin A

5.2.1. Ochratoxin A occurrence in food

Ochratoxin A has been recorded in a very wide range of processed and raw food commodities across the world, but it was first reported in cereal. The occurrence of OTA was recorded in various feeds and food such as cereals, coffee beans, beans, cocoa products, wheat, barley, rice, nuts, wine, dried fruits, meat, and meat products fish, poultry, eggs, milk, spices and medicinal herbs [81, 82]. Also, OTA has been reported in moldy green coffee beans, roasted coffee beans, and coffee brew [83]. Cereals and wine are considered the main contributors to OTA in Europe. Usually, OTA concentrations in processed food are lower than in raw food materials due to the different processing techniques that can cause an effective reduction in OTA level of contamination [84].

5.2.2. Ochratoxin A health effects

The main organ that is influenced by the OTA is the kidney. OTA showed nephrotoxicity to all animal species and is responsible for human renal failure tumors and Balkan endemic nephropathy [85]. OTA has a high affinity for serum albumins and proteins, therefore, when animals consume contaminated feed, these proteins assist in the bioaccumulation of OTA in their organs, resulting in contaminated animal products such as eggs, milk, and other products [86]. Also, OTA is considered teratogenic (cause fetus damages), a probable carcinogen (Causing renal carcinoma), an immune suppressant, and genotoxic causing DNA damage [87]. OTA has been detected in human breast milk and human blood due to dietary exposure [88].

5.2.3. Stability of ochratoxin A in food

Ochratoxin A is relatively heat-stable and most cooking processes to some extent time have not affected their stability [89]. In cereals, the various stages of processing i.e., washing, cleaning, sieving, dehulling, milling, fermentation, and baking have a significant effect on OTA level reduction, as the surface layers that were removed contain a high percentage of fungi and toxins [90]. Reduction in OTA concentration during heat treatments depends on factors such as pH, temperature, moisture, and presence of antioxidants components in a food product [91]. Heating wet wheat at 100 ºC/ 2.3 h cause a 50% reduction in OTA level, while in dry wheat, it took 12 h to give the same reduction. OTA is destroyed by alkaline and acid hydrolysis and by some oxidative agents [92].

5.2.4. Ochratoxin A prevention and control strategies

The ability of OTA producing fungi to grow on a wide range of food products and the relative stability of OTA in the food chain means that the suitable

control point by preventing the crops contamination in the field, at the time of harvesting, drying, and storage of crops by applying HACCP system to prevent the crops contamination [93]. OTA contamination can be managed and monitored, through the following options: good agricultural practices (GAP), good storage practices (GSP), and good manufacturing practices (GMP) such as soil preparation, removal of crop wastes, appropriate planting, fertilizer application, crop rotation, prevent drought stress, crops harvesting in correct moisture content and suitable maturity stage and suitable conditions for crops storage [94].

Pre-harvest and harvest condition are the suitable time to control OTA contamination, but if contamination occurs, the best method is to manage and control infection by post-harvest treatments, include appropriate storage conditions (temperature blow 20ºC and a_W blow 0.70) and use of appropriate packaging material [95]. Monitoring raw materials quality is the most effective control point for processed foods. Any food ingredient that displays visible fungal growth should not use [92]. Besides this, several physicals (separation of contaminated materials), chemical (ammoniation and ozone), Enzymes (both crude and purified enzymes), and biological methods (actinobacteria, bacteria, yeast, and filamentous fungi) can be effective detoxification of OTA [96-99].

5.2.5. Ochratoxin A testing

Many analytical techniques had been used for OTA detection and determination; included (TLC) thin-layer chromatography [100], high-performance liquid chromatography (HPLC) coupled to fluorescence, UV–visible detector [101], and (GC/MS) gas chromatography-mass spectrometry [102]. Although, these methods are accurate but require high costs, time, and complex steps preparation, extraction, purification, detection, and determination. Thus, advanced methods based on biosensors and immunoassays have been developed to overcome these obstacles. Immunoassays are based on antibody-antigen interaction include (ELISA) enzyme-linked immunosorbent assay, fluorescence immuno-assay, (CL-IA) chemiluminescent immunoassay, and (ICA) immuno-chromatographic assay [103]. Also, near-infrared hyperspectral image is an important technique that is applied successfully for fungal infection and ochratoxin A detection in stored wheat and barley [104].

5.2.6. Ochratoxin A legislation

Several countries, especially in Europe, have governmental regulations of OTA concentration in food and feed, include the maximum permissible limits for specific food products. The EU set limits for OTA in cereals, ground coffee, roasted coffee beans, soluble coffee, dried vine fruits, grape juice, and wine. These limits ranged from 2-10 μg Kg^{-1}. The unprocessed cereals limit is 5.0 μg Kg^{-1}, but that for processed cereal products intended for human consumption is 3.0 μg Kg^{-1}. The dried vine fruits limit is 10 μg Kg^{-1}. Also, a limit of OTA is 0.5 μg Kg^{-1} for processed cereal-based food for young children and infants [74]. Switzerland applied a limit of 5.0 μg Kg^{-1} for all food products except cereal-based foods for infants is 0.5 μg Kg^{-1}, Turkey have set a limit between 3.0 and 5.0 μg Kg^{-1} for various food products. Uruguay set a limit of 50 μg Kg^{-1} for cereals, rice, and dried fruits and Canada sets a limit of 2000 μg Kg^{-1} for OTA in poultry and pig feed. Egyptian standard set limits of 5 μg kg^{-1} for OTA in unprocessed cereals, roasted coffee beans, and ground roasted coffee. OTA limit in dried vine fruits and soluble coffee is 10 μg kg^{-1} and 2 μg kg^{-1} for grape and wine. While, the limit of OTA for processed cereal-based food and baby foods for infants and young children and dietary foods for special medical purposes intended specifically for infants is set as 0.50 μg kg^{-1} [75].

5.3. Fumonisins

Fumonisins are secondary toxic metabolites produced by certain fungal species of the genus *Fusarium*, which grown in cereals. More than 15 analogs of fumonisin have been identified and characterized into five groups as fumonisin A, B, C, P, and H according to the chemical structure [105]. Fumonisins are polar compounds based on the long-chain hydroxylated hydrocarbon that contain methyl- and amino- groups. Fumonisin B group (FB1, FB2, FB3, and FB4) are the most abundant fumonisins in nature, and FB1is the most important and most toxic form. The molecular weight of FB1 is 721 g/mol and its chemical formula is $C_{34}H_{59}NO_{15}$ **(Figure 4)**. Fumonisins are mainly produced by *F. proliferatum* and *F. verticillioides*. However, some species also reported to produce fumonisins like *F. nygamai*, *F.*

napiforme, *F. anthophilum*, and *F. dlamini* and are associated with the food grains [106].

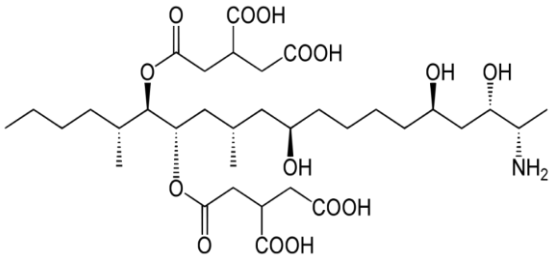

Fig. 4. Fumonisin B_1

5.3.1. Fumonisins occurrence in food

Maize and maize-based products are the most common group in foods contaminated with fumonisins, as well, cereals like rice, sorghum, wheat, barley, oat, millet, and rye [107]. FB1 has been reported to contaminate several food products such as garlic, barley foods, beers, asparagus, dried figs, and milk. However, contamination with FB1 and FB2 is reduced by 59% during the manufacturing of chips from maize flour due to the heat treatment [108]. Further, several products like Portuguese maize bread, cornflakes, tea (black and herbal), and some medicinal plants have also been reported to contaminate by fumonisins [109].

5.3.2. Fumonisins health effects

Exposure to FB1 expresses both acute and chronic toxicity symptoms in an infected animal. The target organs that are affected by fumonisin are the liver and kidney and the infection severity depending upon the species and strain [110]. The intestine is possible to target organs for fumonisin toxicity. Fumonisins in moldy feed can be associated with some livestock diseases, especially pigs and horses. Horse´s long-term consumption of feed contaminated with fumonisins cause a fatal disease called equine leucoencephalomalacia (ELEM), which leads to degeneration in the brain, neurotoxic effects, and liver damage [10]. The long-term chronic toxicity of FB1 showed an adverse effect on kidneys and liver of mice and rats and at high levels of FB1 caused carcinogenicity. Also, FB1 is the causative agent for esophagus and liver cancers in humans [111].

5.3.3. Stability of fumonisins in food

Fumonisins are relatively heat-stable and noticeable destruction only occurs when the temperature reached above 150 – 200ºC during food processing such as frying, roasting, baking, and extrusion cooking. The reduction percent in fumonisin level depends upon cooking conditions and the food matrix composition [112]. Also, the reduction can be formed due to the conjugate of fumonisins with other food components causing structural modifications of fumonisins [113]. Interaction of FB1with reducing sugars leads to the strong covalent bond formation during heat treatments. For example, FB1 reacts with corn grits D-glucose during extrusion cooking at 160-180ºC forming N-(deoxy-Dfructos-1-yl) FB1 [114]. The wet milling caused a significant reduction of fumonisins content when compared with the dry milling process which causes a negligible decrease in fumonisin content [115]. FB1 content in beer has not been affected by brewing and fermentation processes.

5.3.4. Fumonisins prevention and control strategies

Implementation of good agricultural practices (GAP) system leads to reduce *Fusarium* infection of cereal crop and also effective in reducing the fumonisins formation. Also, using genetic engineering and nanotechnology to develop resistant varieties of crops for *Fusarium* infection and fumonisins contamination, good storage practices (GSP) by rapid dry and reducing the moisture content (a_W value 0.8 immediately after harvest), and good manufacturing practices (GMP) can also mitigate fumonisins contamination [116]. Physical decontamination methods like screening, separation, pyrolysis, irradiation, and milling process can be more effective in fumonisins levels reducing in contaminated maize. Chemical detoxification can be performed by alkaline *e.g.*, ammonia, sulfur dioxide, and sodium hydroxide treatments and oxidation like ozone [117]. Biological control such as seed treatment with *Microbacterium oleovorans* and *Bacillus amyloliquefaciens* could decrease FB1 and FB2 in maize grains, also *Azotobacter*, *Bacillus*, *Pseudomonas* and *Arthrobacter* reduced *F. verticillioides* infection and FB1 production [118-120].

5.3.5. Fumonisins testing

The traditional analytical chromatographic techniques used for detection and quantification of fumonisin include thin-layer chromatography (TLC), HPLC, and ultra-high performance liquid chromatography (UHPLC) coupled with a fluorescence detector (HPLC-FLD), UV-Vis spectrophotometry, and liquid chromatography-mass spectrometry are currently used (HPLC-MS) [93, 121]. These methods are accurate but require time, expensive, and need complex steps of extraction, purification, detection, and determination [122]. Recently, molecular techniques were applied to detect fumonisins producing strains like the multiplex PCR technique that used to detect fumonisins producing *F. verticillioides* strains [123] and PCR-ELISA for detection of *F. verticillioides* in corn-based on FUM21 gene [124]. In addition, indirect competitive ELISA and nano-gold-based gray imaging quantification immunoassay (GNPs-GI) have been used to detect FB1 in agricultural products [125].

5.3.6. Fumonisins legislation

Very few countries outside North America and Europe have introduced the guideline levels or permissible limits for fumonisins in foods. The EU set 4000 μg Kg^{-1} as a maximum limit for total FB1 and FB2 in unprocessed maize, while 1000 μg Kg^{-1} and 800 μg Kg^{-1} were set for maize and maize-based food prepared for direct human consumption and snacks and maize-based breakfast cereal, respectively . Whilst the maximum limit for maize-based food for young children and infants is 200 μg Kg^{-1} [74]. USA applied a varied limit from 2000 to 4000 μg Kg^{-1} for FB1, FB2. Egyptian standard set maximum levels of 4000 μg kg^{-1} for the sum of FB1 and FB2 in unprocessed maize; from 1400-2000 μg kg^{-1} milling fractions according to particle size, 1000 μg kg^{-1} for maize intended for direct human consumption, 800 μg kg^{-1} for maize-based breakfast cereals and maize-based snacks and 200 μg kg^{-1} for processed maize-based foods for infants and young children [75].

5.4. Patulin

Patulin, 4-hydroxy-4,6-dihydrofuro[3,2-c]pyran-2-one **(Figure 5)** is a water-soluble polyketide secondary metabolite produced by a variety of filamentous fungi like *Aspergillus*, *Bysochlamys*, *Penicillium*, *Peacylomyces*, and *Eupenicillium*. Patulin was first described as an antimicrobial active compound due to its strong activity against several gram-negative and gram-positive bacteria including *M. tuberculosis* [126]. In addition, the antibacterial, antiprotozoal and antiviral activity of patulin, was toxic to animals and human, these problems prohibiting clinical use of patulin as an antibiotic [127]. Several studies reported the toxicity, immunotoxicity, and mutagenicity of patulin, and classified it in group 3 by International Agency for Research on Cancer (IARC) as a carcinogenic agent in humans and animals [128, 129]. Consumption of food contaminated with high levels of patulin can cause serious troubles to human health, especially the children. Acute symptoms of patulin as convulsion, perturbation, edema, ulceration, intestinal inflammation, vomit, and nausea were observed after ingestion of patulin contaminated food [130].

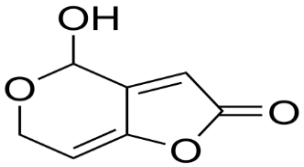

Fig. 5. Patulin

5.4.1. Patulin occurrence in food

Patulin is mostly found in apples, apple juices, and jams. It was also reported in other fruits such as pears, grapes, cherries, bananas, plums, blueberries, pineapples, oranges, strawberries, peaches, figs, watermelons, and apricots [131]. Patulin was also detected in cereals like wheat, barley, corn, rice, peanuts, oats, cottonseed, and legumes [132]. Interestingly, patulin was recorded in baby food, milk, cheeses, seafood (fish and shellfish), meat, some vegetables, and poultry feed [133, 134]. The most source contributor to patulin in the diet are considered apples and apple products, many studies have reported patulin contamination in apple juices in several countries, such as Canada, France, Italy, Austria, Sweden, Spain, UK, USA, Australia, Japan, Iran, Turkey, Brazil and South Africa [135].

5.4.2. Patulin health effects

Most information about the toxicity of patulin is derived from animal studies and there is little or no data about chronic or acute toxicity. Patulin has a

high toxic effect on kidneys, liver, immune system, and gastrointestinal tract. Also, it is reported as a risk factor for genotoxicity, teratogenicity, neurotoxicity, cytotoxicity, immunotoxicity, and carcinogenicity [136]. Acute toxicity is reported in animals with symptoms like convulsions, agitation, dyspnea, edema, pulmonary ulceration and congestion, hyperemia, and distension of the gastrointestinal tract [137]. Sub-acute signs like intestinal and changes weight loss, and renal function alterations were observed in the rat after patulin ingestion. Also, the same clinical symptoms were reported in mice, hamsters, and chickens [138]. Longer-term chronic effects are considered of more concern from a food safety point of view. Patulin is classified in group 3 by International Agency for Research on Cancer (IARC) as a carcinogenic agent in humans and animals [128].

5.4.3. Stability of patulin in food

Patulin is considered an important problem only in apple and apple juice. Patulin is relatively heat-stable and is not destroyed by apple juice pasteurization (90ºC for 10s), heat treatment of apple juice at 90 and 100°C/20 min observed reduction patulin content of 19 and 26%, respectively [139]. Patulin is a water-soluble molecule, washing apples with water under high pressure reduced patulin content between 10 to 100% [140]. High hydrostatic pressure (non-thermal food process method) has been reported to be effective in patulin removal from apple juice [141]. Another choice, sulfur-containing compounds can destroy patulin, sulfur dioxide (200 ppm) was capable of reducing 90% of patulin content in apple juice within two days [142]. Also, immersion of apples in sodium hypochlorite 3% (5min/25°C) completely inhibited fungal growth and reduce patulin content [143]. Clarification and filtration of apple juice with granular activated carbon has a great effect on patulin removal (PAT levels reduce patulin level by about 98%), but this process harms some quality characteristics of apple juice like reducing the color and phenol content [144].

5.4.4. Patulin prevention and control strategies

To avoid patulin contamination in food products, the control system should be implemented throughout the product chain like prevent fungal and insects infection, avoid the physical damages caused by poor handling during harvest, transportation, and storage, which allows the pathogenic fungi to infect fruits and cause spoilage [145]. Droughts, rainfall, humidity, and insect infection are the optimal conditions for fungal growth in fruits and patulin contamination. Patulin has relatively resistant to different fruit juices processing. Therefore, the application of good pre- and post-harvest practices could limit the growth of toxigenic fungi and subsequently patulin production. Good agricultural practices (GAP) in fruits production, good storage practices (GSP) by controlling the storage parameters (humidity, temperature, O_2, and CO_2 content) to prevent fungal in storage fruits [146] and Good manufacturing practices (GMP) during juice manufacturing like pressure washing, sorting, remove damaged fruits and quality inspection of fruits before processing are the most effective practices to minimize fruit contamination by patulin [147].

The major amount of patulin is removed or degraded from the fruit during processing, i.e., grading and sorting, washing, depectinization, filtration, pasteurization, and fermentation [148]. Several methods are used for the removal or degradation of patulin from fruit juices. Physical treatments such as heat treatments, ultraviolet radiation, pulsed light, and high hydrostatic pressure [149]. Chemical additives such as ascorbic acid, potassium permanganate, ammonia, sulfur dioxide, and ozone are used for patulin degradation [150]. All of the following biological agents were applied for patulin reduction; microorganisms like lactic acid bacteria (*Lactobacillus brevis*, *Lactobacillus plantarum*, *Bifidobacterium animals*, and *Enterococcus faecium*), yeast such as *Sporobolomyces* sp., *Kodameae ohmeri*, *Saccharomyces cerevisiae* and filamentous fungi as *Byssochlamys nivea*; as well as enzymes, *e.g*., lactone degrading enzyme (β-lactamase) [151-153].

5.4.5. Patulin determination

The most suitable methods of analysis for patulin detection and determination in food products, especially apple, apple juice, and jams with high accuracy, low limits of detection, low limit of quantification, and simple producers includes the chromatographic techniques like thin layer chromatography (TLC) with advantages of simple procedures and low cost, but the high limit of detection (LOD) as 20 μg L^{-1} [154]. High-performance liquid chromatography (HPLC) coupled

with (UV) or photodiode array (DAD) detector is the most used technique for patulin determination; easy for patulin identification and quantification with the LOD value 5 μg L^{-1} [151]. HPLC associated with mass spectrometry (HPLC-MS) recorded lower LOD value 4 μg L^{-1} [155]. Gas chromatography (GC) and GC-MS used to patulin analysis by electron impact ionization using silylated patulin derivative and raw patulin were also used [156]. Alternative methods like capillary electrophoresis (CE) are also used for patulin analysis as a highly sensitive and rapid analysis of patulin in apple juice [157].

5.4.6. Patulin legislation

The maximum level of patulin in food, especially in apple juice, has been regulated in several countries. The European Union (EU) has set a maximum limit of 50 μg Kg^{-1} for patulin in fruit juice and drinks contain apple juice or its derivatives and 25 μg Kg^{-1} for solid apple product like apple puree, while a low limit (10 μg Kg^{-1}) has been set for apple-based foods prepared for infants [158]. FDA has set 50 μg Kg^{-1} as an upper limit for patulin in apple juices and concentrates apple juice. The Egyptian standard has set 50 μg kg^{-1} as a maximum limit for fruits juices, concentrated fruits juices, fruits nectars, spirit drink, cider, and other fermented drinks derived from apple or containing apple juice. The limit of 25 μg Kg^{-1} was set as the maximum limit for a solid apple product, including apple puree and apple compote intended for direct consumption and 10 μg kg^{-1} maximum limit for apple juice and solid apple products, including apple puree and apple compote for infants, young children and baby foods other than processed cereal-based foods infants and young children [75].

5.5. Zearalenone

Zearalenone is a toxic fungal metabolite produced by certain fungal species of genus *Fusarium*, *e.g.*, *F. graminearum* (*Gibberella zeae*), *F. verticillioides*, *F. cerealis*, *F. culmorum*, *F. crookwellense*, *F. equiseti*, and *F. semitectum*, which are distributed all over the world and cause cereals grains infections in field and during storage [14]. Zearalenone or 6-(10-hydroxy-6-oxo-trans-1-undeceny) β-resorcylic acid lactone **(Figure 6)** has a molecular weight of 318.4 g/mol and a molecular formula of $C_{18}H_{22}O_5$ [159]. Zearalenone has estrogenic effects, these acts of estrogen hormone cause several reproductive troubles in domestic animals (especially sheep and pigs) and hyper-estrogenic syndromes in humans, which depend upon the time of exposure and the dose [160]. High zearalenone concentrations can cause symptoms of vomiting, nausea, and diarrhea which are associated with cereal toxicosis due to incorrect storage conditions rather than due to production processes [161]. Several related molecules of zearalenone were identified in a fungal culture like α and β- zearalenols, but the presence of these compounds in food is uncertain [162].

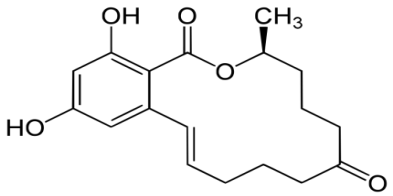

Fig. 6. Zearalenone

5.5.1. Zearalenone occurrence in food

Zearalenone has been recorded all over the world in cereals, including maize, barley, wheat, rice, sorghum, oats, some legumes, and other crops. A high level of zearalenone has also been found in Indian bananas [159]. The levels of zearalenone in feed and food vary over a wide range depending upon the climatic conditions, which are the most effective factor in zearalenone level of contamination in cereals crops. Zearalenone has been also found in processed food, especially those derived from cereals such as corn and wheat flour, breakfast cereals, bread, snacks, biscuits, noodles, and corn beer. Zearalenone may be excreted from contaminated grains into beer at the different stages of the brewing process [163]. Zearalenone may be biotransformation into the metabolite β- zearalenols in beer during yeast fermentation. Also, zearalenone can be excreted into milk due to fed lactating animals on feeds contaminated with high levels [164]. Zearalenone cannot be detected in processed meat due to unsuitable conditions for fungal growth and toxin production. While, zearalenone was recorded in chickens' muscles, fat, and egg which were fed by contaminated feeds for a long time [165].

5.5.2. Zearalenone health effects

Zearalenone has strong genotoxic and cytotoxic activity, but the main threat to animal and human health through causing estrogenic disruption [166]. Zearalenone exhibited strong estrogenic activity, after entre to the bloodstream it causes reproductive problems in several animal species, especially sheep and pigs like problems miscarriage and increases the risk of polycystic ovary progression [167]. Natural exposure to contaminated food with zearalenone has been recorded as a causative agent to female reproductive organ changes and cervical cancer [168]. Also, zearalenone exhibits apoptosis, strong embryonic toxicity, and oxidative stress in embryonic stem cells of humans [169]. Exposure of zebrafish embryos to zearalenone induces developmental defects like pericardial edema, reduction in heart rate hyperemia, spine curvature, and yolk sac edema [170]. Consumption of food contaminated with high levels of zearalenone can cause symptoms of vomiting, nausea, and diarrhea [161].

5.5.3. Stability of zearalenone in food

Zearalenone is a heat-stable molecule, no change in its structure was observed after heat treatment at 120ºC/4hr [171]. At 200ºC, zearalenone levels decrease 37% and 69% by time increasing from 30 min to 60 min, respectively. In addition, decrease in zearalenone levels 34-40% due to baking of dough 190-200°C into bread, 48-62% during the manufacture of instant noodles, and 16-27% during biscuits preparation [172]. Roasting of contaminated corn at 110-140°C decreased zearalenone levels by 50% [173]. Yellow corn fermentation using *Saccharomyces uvarum* for 5 days/32°C showed little decrease in zearalenone concentration. While fermentation using lactic acid bacteria has shown a significant decrease in zearalenone levels [164]. Several efforts have been made to decrease zearalenone levels in contaminated crops using several chemicals. Treatments of ammoniation or use hydrochloric acid, propionic acid, sodium bicarbonate, acetic acid, and hydrogen peroxide were not effective in zearalenone reducing the level decreasing contaminated yellow com [14]. However, destruction of zearalenone (96-100%) in both natural and artificial contaminated corn grains was achieved using both liquid and gaseous formaldehyde. Also, the destruction of zearalenone was reported when the aqueous solution of toxin treated with ozone 10% for 20 sec. [69].

5.4.4. Zearalenone prevention and control strategies

Since the production of zearalenone occurs in the field and during the storage stage. So, the control system should be applied in both pre-harvest and post-harvest stages. Good agricultural practice (GAP) which designed to reduce the infection of cereal crops by *Fusarium* spp. are also effective in limiting zearalenone contamination. These control options include land preparation, crop waste removal, and crop rotation to reduce the microbial load of *Fusarium* in the field; breeding fungus-resistant crop varieties; using the effective fungicides in a suitable time, harvesting crops at the correct stage of maturity and suitable moisture content [14]. Good storage practices (GSP) control the storage parameters (humidity and temperature) to prevent fungal in cereal crops during the storage stage. Several techniques have been developed to decontamination of zearalenone like physical decontamination, e.g., gravity separation that can be effective in reducing zearalenone concentrations in contaminated grains, milling process also able to reduce zearalenone levels in grits and corn flour by 80-90% and heat treatment which usually not effective. Chemical methods, such as ammoniation, or use hydrochloric acid, acetic acid, and hydrogen peroxide, formaldehyde, and ozone were used for zearalenone reduction [69]. Biological agents such as yeast-like *Saccharomyces cerevisiae* and lactic acid bacteria such as *L. rhamnosus* and *L. plantarum* were applied to zearalenone detoxification [174].

5.4.5. Zearalenone testing

Zearalenone can be determined in foods and feeds by different analysis techniques. Thin-layer chromatography (TLC) was the traditional and most popular technique for mycotoxins analysis. High-performance liquid chromatography (HPLC) equipped with various detectors, *e.g.*, fluorescence detector (FLD), UV detector (UV-Vis or PDA), and electrochemical detector (EC), as well as high-performance liquid chromatography-mass spectrometry (HPLC-MS), were also used to detect the presence of zearalenone and its metabolites in body fluids and feces [175]. HPLC-MS/MS was used to analyze the toxin in food, biological and

environmental samples [176]. Also, gas chromatography-mass spectrometry (GC-MS) was used to zearalenone and its metabolites analysis by derivatization before chromatography as a sample of silylating agents [177]. Immunoassay methods were used for zearalenone detection, such as enzyme-linked immunosorbent assay (ELISA) [178] and lateral-flow immunochromatographic assay (ICA) [179].

5.5.6. Zearalenone legislation

Few countries outside the European Union have introduced the guideline levels for zearalenone. The EU sets the maximum limit for zearalenone in most unprocessed cereals as 100 μg Kg^{-1}, while, unprocessed maize is 350 μg Kg^{-1}. In maize prepared for direct human consumption maize-based snack and cereals 100 μg Kg^{-1} stated as a maximum permissible limit while that 75 μg Kg^{-1} maximum levels set out for other cereals like bran and flour for direct human consumption. Whereas, the limit for bread, cereal biscuits, snacks, pastries, and breakfast cereal is 50 μg Kg^{-1}. The limit for foods intended for young children and babies is 20 μg Kg^{-1} [74]. Chile has set 200 μg Kg^{-1}as the maximum limit for zearalenone for all foods, Indonesia requires zearalenone to be not detected in maize, Iran has set 200 μg Kg^{-1} as a limit for most cereal and Canada has introduced 3000 μg Kg^{-1} as a permissible limit for zearalenone in pig feeds. The Egyptian standard has set the permissible limit of maize prepared for direct human consumption maize-based snack and cereals is 100 μg Kg^{-1} while 75 μg Kg^{-1} set out for cereals intended for human consumption, cereal bran, and flour and germ as end product marketed for direct human consumption. The limit for bread (including small bakery wares), biscuits, cereal snacks, pastries, and breakfast cereal is 50 μg Kg^{-1} and 20 μg Kg^{-1} for processed cereal-based foods for infants and young children [75].

Conclusion

- The growth of fungi in food may be a serious indicator for the presence of severe danger to the consumers due to mycotoxins production.
- Mycotoxins can contaminate different types of food like cereals, fruits, vegetables, and processed foods.
- Once mycotoxins contaminate foods it is very difficult to remove them from foods because mycotoxins are very stable molecules. So, control strategies should be established to monitor and prevent mycotoxins contamination during pre-harvest and post-harvest stages.
- Different methods were used for mycotoxins determination in food and feed such as TLC, HPLC, HPLC-MS, HPLC-MS/MS, and GC-MS.
- Several countries around the world set legislation to regulate the maximum limits of mycotoxins in food and feed.

Conflicts of interest

The authors declare no conflict of interest.

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