






Occurrence, identification, and decontamination of potential mycotoxins in fruits and fruit by-products

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Abstract

The incidence of aflatoxins, ochratoxin A, and patulin in fruits and processed fruit products has been ever more challenging and gained additional focus on ecofriendly mitigation strategies. The onset of these toxins is due to several factors involving insect attacks, agricultural practices, and climate change. Acute and chronic health hazards are clinically proven after consuming contaminated foodstuffs, even at lower concentrations of mycotoxins. Synergistic, masked, and substantial occurrence in fruit matrices increase their complexity in detection and detoxification; apparently, this article reviewed the available information on the occurrence of mycotoxins in several fruits and their products, focused on the conventional and advanced methods of identification, quantification, and decontamination techniques. Strengthening and implementing stringent international and national guidelines are required for impactful, tangible measures in the future. Nevertheless, controlling the mycotoxins in fruits will certainly be challenging for scientists. Therefore, more impactful technologies are still needed to eliminate the toxins at the threshold level of the food chain and ensure sustainable global food safety.

KEYWORDS

aflatoxin, alternaria, citrinin, decontamination, fruits, identification, mycotoxin, ochratoxin, patulin

1 | INTRODUCTION

An apple a day keeps a doctor away and tells all about the importance of fruits in our lifestyle. However, fruits are highly susceptible to infection

by toxic molds and microorganisms. Mycotoxins have gained critical attention and importance globally due to their impact on human health, agricultural practices, processing and preservation industries, economic loss, and food chain management. Mycotoxins are traced more

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often in cereals, which include aflatoxins (AFs), ochratoxin A (OTA), and patulin (PAT), produced by *Fusarium* species. In contrast, a cluster of mycotoxins, including tenuazonic acid (TeA) attenuates, altertoxin I and II, alternariol (AOH), alternariol methyl ether (AME), are produced by *Alternaria* species (Bangar et al., 2022; De Souza et al., 2021; Heshmati et al., 2021; Jafari et al., 2021). Generally, mycotoxin contamination causes greater economic losses. On a comprehensive view, AFs are detected in nuts, dried fruit, and spices, while *Fusarium* and OTA are identified in cereals, particularly maize, wheat, and barley. However, PAT and citrinin (CIT) are identified from fruits and processed products (Drusch & Aumann, 2005). Toxigenic fungi infect various agricultural and horticultural crops during the products' growth, storage, harvest, and processing (Mokhtarian et al., 2020; Pires et al., 2022; Khaneghah et al., 2023). The incidence of fungal growth depends on several physiochemical factors; besides, the water content in fruits boosts the rapid mycelial growth; however, the pH of natural acids like citric, malic, and tartaric acids (Fernández-Cruz et al., 2010; Jiang et al., 2021;) has less impact on the mycotoxin fungi. The early infestation of fungi in fruits leads to mycotoxin, even in fruit juice. This is due to the resistance of the fungi against high temperatures and pressure. The major toxins studied in fruits and fruit products include AFs, OTA, PAT, and *Alternaria* toxins such as AOH, CIT, AME, and Altenuene (ALT) (Iqbal et al., 2018). However, PAT is most widely studied in processed fruit products. In addition, toxins from AOH, AFs, and OTA have also been reported (Mandappa et al., 2018). Therefore, it is necessary to monitor multimycotoxins' presence in processed fruit products (Iqbal et al., 2018; Joshi et al., 2022). The onset of common rot or mold infections in the fruiting plants paves the way for entering the mycotoxins into the food web. In citrus fruits, rot disease is caused by *Penicillium italicum*, *P. digitatum*, *Geotrichum candidum*, *Alternaria alternata*. While the species of *Aspergillus* cause brown rot infection in the plant, enabling mycotoxins to enter. Similarly, in the case of melons, the infection is caused by *P. expansum* and *Botrytis cinerea*. While in the case of other fruits like stone fruits, figs, grapes, and berries are also infected by *Aspergillus*, *Mucor*, and *Fusarium* species (Logrieco et al., 2003; Kahramanoglu and Usanmaz, 2021). Figure 1 demonstrates the causes, types of molds, and their corresponding mycotoxins produced in fruits. More focus on the Hazard Analysis Critical Control Point (HACCP), preharvest and postharvest strategies, processing, and preservation should be monitored. A recent review implied the comparative effects of physical, chemical, edible-film packaging, and other antagonists technology in controlling mycotoxigenic fungi in fruits and vegetables (You et al., 2022). High carbon dioxide and low oxygen levels support the fungi's growth and metabolism. Further specific genes like the creA gene are pertinently associated with modifying the carbon sources found in the surrounding environment, often called carbon catabolite repression, that augments the growth of fungi. In parallel, it regulates the nitrogen assemblage, termed nitrogen metabolite repression. These genes play vital roles in producing fungal metabolites, virulence, morphology, and adaptation of the mycotoxigenic fungi. However, the nmrA gene is also found to regulate the transcription in nitrogen metabolism. The pH of the environment triggers the expression of AopacC, which in turn produces OTA; studies have proven the mechanism of the AopacC gene

through a knockout mechanism which curtailed the production of OTA (Wang et al., 2018). Physical and chemical methods of decontamination were widely reported. However, adopting microbial antagonists has distinctive cues for preservation with eco-friendly effects. Effective findings include the application of *Rhodosporidium paludamentum* (Lu et al., 2022), *Cryptococcus laurentii* (Zhang et al., 2022), and *Pantoea agglomerans* strain EPS125 (Bonaterra et al., 2003) in control fungal rots in fruits were reported. Indeed, these microbial antagonists have high market value compared with chemical fungicides. Therefore based on the literature evidence, a wise choice in the selection of combination methods has been experimentally proven to mitigate this toxin production by fungal species. Low temperature fades the gaseous metabolism in fruits which effectively halts the expansion of the microbial population. Active packaging and modified preservation methods delay fruit ripening through controlled air discharge. In contrast, irradiation methods are effectively practiced in killing the microorganisms found superficially on the surface of fruits. Experiments have proved that natural or chemically synthesized bacteriostatic agents are of great significance to the control of mycotoxins and combining them with edible membranes has also been favored. Further, a combination of bacteriostatic agents with edible film coating has also been successful. These complex methods aid in controlling fruit decay factors and inhibit the expansion of fungal colonies. On the other end, simple fresh-keeping methods should also work hand-in-hand to bring up a healthy practice from farmlands to cold storage shelves. Hence, based on the discussed interventions, the present review addressed the types of mycotoxins identified in fruit and fruit-based products, their significance in the food industry, detoxification methods, and critical mitigation strategies to control mycotoxin.

2 | MYCOTOXINS IN FRUIT AND FRUIT PRODUCTS

2.1 | Citrinin

The dominant fungal species, like *Penicillium*, *Aspergillus*, and *Monascus*, reported producing CIT (Ostry et al., 2013). It affects cereal crops, foodstuffs, and fruits, in almost all climatic conditions. The fungi affect the food commodities preharvest and postharvest and contaminate the harvested and stored grains, spices and condiments, citrus fruits, herbs, and processed fruit juices. In addition, CIT is detected in oranges, sweet cherries, and tomatoes (Wang et al., 2016), commercial beers, dried grapes, grapes and pears, lager beer, and apples (Pepeljnjak et al., 2002). The CIT production is increased by other factors such as oxygen availability, carbon sources, humidity, temperature, storage, and the addition of preservatives (Dohnal et al., 2010). However, the safe limit in the food matrices varies throughout the countries. For instance, the maximum limit of CIT in foodstuffs was 50, 200, and 2000 μg in China, Japan, and European Union in 2014, respectively (Urraca et al., 2016). The CIT-mediated harmful effects include oxidative stress, altered antioxidative responses, promoted the generation of oxidative stress, and heightened the synthesis of

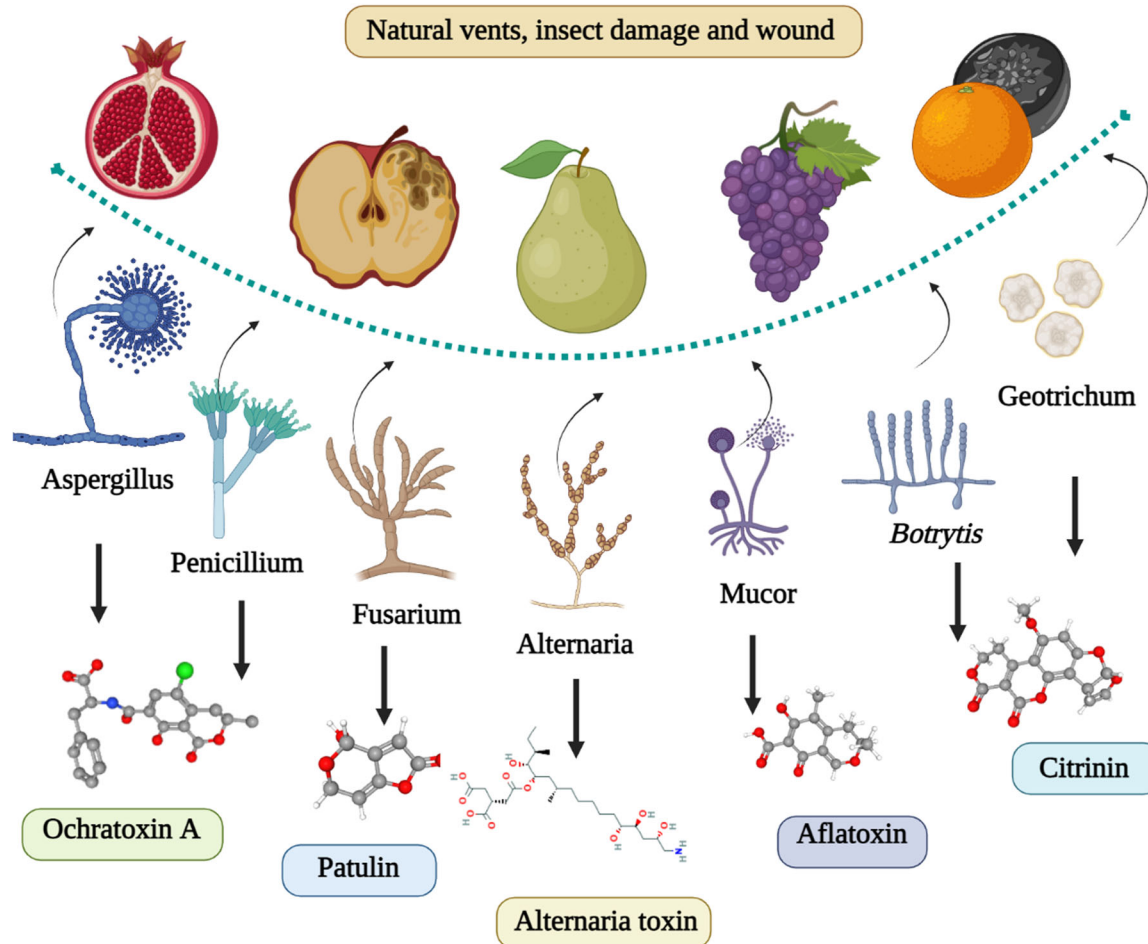


FIGURE 1 Outline on the occurrence, type of common molds, and the mycotoxins affecting fruits

superoxide anions. They predominantly lead to malfunctions associated with mitochondria dysfunction, lipid peroxidation, and cell death (Yu *et al.*, 2006). The CIT is more likely associated with other potential mycotoxins like OTA, AF B, and PAT, commonly detected in fruit and fruit products like apple juices and jams (EFSA, 2014). The detection, identification, and quantification methods include basic and advanced chromatographic techniques involving TLC, HPLC, LC-MS, ELISA, capillary zone electrophoresis, and immunochromatographic assay. Degradation of CIT has been successful through integrative techniques involving physical, chemical, biological, and nano-based approaches. However, curtailing the causative organisms in the early stages of intervention into the food web seems to be the best mitigation strategy. The practice of HACCP, good agricultural practices, and good manufacturing practices has been proven to be effective in regulating the CIT levels in raw agriculture products. The traditional molasses in Turkey were reported to have high concentrations of CIT and is prepared using the raw fruits of grape, mulberry, fig, and apple (Oztas *et al.*, 2020). Using rotten fruits and low-quality processes induces the incidence of other mycotoxins and CIT. A similar study supported the same fact and a maximum level of 0.2 $\mu\text{g}/\text{L}$ in fruit and vegetable juices (Dietrich *et al.*, 2001)

2.2 | Aflatoxin

Aflatoxin contamination in fruits commonly occurs in temperate regions. It is often reported in citrus fruits (Drusch & Ragab, 2003), apple juices (Abdel-Sater *et al.*, 2001), dried figs (Senyuva *et al.*, 2007), red grape juices (Scott *et al.*, 2006), red and white wines (Bellí *et al.*, 2004), blueberries, strawberries, raspberries (Drusch & Ragab, 2003) pear, and apple marmalades. The detection limits of AF in juices range between 0.01 (Visconti *et al.*, 2008) - 39.42 $\mu\text{g}/\text{L}$ (Karaca & Nas, 2006), while in the case of raw fruits and vegetables, the limits vary between 0.21 and 11,300 $\mu\text{g}/\text{kg}$ (Drusch & Ragab, 2003). The AFB1 is observed to be widely present in a concentration between 2 and 550 g/kg in fruit commodities like dried grapes from Brazil, Egypt, Greece, India, and Morocco (Juan *et al.*, 2008). Combinations of mycotoxins like OTA along with AFB1 were also reported in fried figs from Turkey, up to 63 g/kg (Senyuva *et al.*, 2005). However, the detection limits of AFB1 and other components like AFB2, AFG1, and AFG2 in certain foodstuffs such as cereals, nuts, and dried fruits are set, but the maximum levels were not set in fruit juices (EC, 2006). Fungal isolates were compared in fresh and dried persimmon fruits. The dominant microflora was *Rhizopus* sp., *Penicillium* sp., and *Aspergillus* sp. (Gündüz *et al.*, 2020). Due

to prolonged exposure to the AF toxin in fruits, its presence has been extended in fruit-based food matrices like fresh orange juices, pineapple, peach-packed juices, and apple and guava juices (Pallares et al., 2021). A recent study unveiled that the chronic incidence of AF toxins in these food products has increased the risk factor in young people (EFSA, 2020) due to their dietary exposure and lifestyle. Intrinsic elements like moisture content, pH, redox, osmotic pressure, enzymes, nutrient composition, and inhibitors promote the growth of mycotoxin fungi. Furthermore, abiotic factors like C/N, light, temperature, and humidity influence AF biosynthesis (Ehrlich et al., 2010). The mycological profile of the date fruits in local markets of Saudi Arabia was studied, and they exhibited the presence of AFs and OTA due to the infestation of *A. flavus*, *A. niger*, and *A. terreus* (Gherbawy et al., 2012). In a recent study, AF contamination due to *A. parasiticus* and *A. flavus* L. was identified in the baobab fruit in Kenya. The toxin concentration was due to poor postharvesting practices (James et al., 2022). Certainly, dry fruits like dates, pistachios, and walnuts are more prone to AFB1, AFB2, AFG1, and AFG2 contamination at all stages, from harvest to marketing. However, a recent study reported that the margin of exposure determinations of the dry fruits stored at 4°C and closed glass containers curtailed the growth of the mycotoxigenic mold in South Punjab, Pakistan (Naeem et al., 2022). In a similar study, the AFs content in pekmez was analyzed to be 7.5 µg/kg of AFB1, 1.5 µg/kg of AFB2, G1 (AFG1), and G2 (AFG2), however after clarification, the levels decreased by 60.4, 76.7, 76.3, and 86.7%, respectively (Heshmati et al., 2019). The presence of AFs was identified in fruit chips samples marketed in Tabriz City, Iran, and was quantified through a liquid-liquid extraction procedure (Mohebbi et al., 2022).

2.3 | Patulin

Patulin is another significant mycotoxin produced by diverse mold forms like *Byssoschlamys* sp, *Aspergillus* sp, and *Penicillium*. They are often detected in overripe fruits, especially apples, cereals, and vegetables. Generally, PAT occurs synergistically along with other mycotoxins and produces adverse health impacts like immunotoxicity, mutation, and genotoxicity and causes impactful damage in the gastrointestinal parts of rodents (Welke et al., 2009). Their concentrations are the key factors for determining the index on the stage of a rotten apple in the manufacture of apple juices in a large-scale setup. The Joint Expert Committee on Food Additives suggested the tolerable upper limit of PAT to be 0.4 µg/kg body weight/day (Joint, 1996). However, in most countries, there is no provision for critical standards followed in fruit and its products. Unhygienic handling and unfavorable processing methods influenced persistent PAT toxicity in fruit-based canned products, bottled beverages, and sundried and shelled products. Presently, the permissible limit of PAT is 50 µg/kg in fruits and their derived products (Commission Regulation, EC, 2006; GB 2761e2011). Detection of PAT is more challenging due to its low molecular weight, and its immunotoxicity is more destructive if it is synergistically present in combinations with other mycotoxins (Vidal et al., 2019). Effective reduction of PAT in apple juices was noted after subjecting to a series of

physical protocols such as pasteurization, enzymatic treatment, micro-filtration, and evaporation processes. According to Codex Alimentarius Commission, PAT content below 50 µg/L in apple juices was considered the lower limit. However, 10 µg/L of PAT is suggested to be a safer limit intended for infants and children (Welke et al., 2009). It is familiar that apple and apple products are highly affected by PAT. However, the other fruit-based food like juices, smoothies, and salads showed 182 µg/kg of PAT in a study reported from Pakistan (Iqbal et al., 2018). Occurrence of PAT in baby foods marketed in Italy was detected up to 5.23 ng/ml. Further the concentration of PAT in organic products was less up to 0.13 ng/ml than the conventionally grown tomatoes, which ranged up to 1.92 ng/ml (Sarubbi et al., 2016). In a similar study, the PAT levels were comparatively studied in the apples and tomatoes cultivated by conventional and organic farming methods. Other causative fungal species were *Rhizopus*, *Mucor*, *Alternaria*, *Cladosporium*, *Botrytis*, *Aspergillus*, and *Penicillium*, identified through DNA barcoding. However, no significant difference in the levels of PAT concentrations was noted among the organic and traditional cultivars. Notably, the highest concentration of PAT was recorded in the delicious golden variety of apples than in the reineta and fuji apples. Furthermore, commercial tomato products showed up at 3.22–47.72 µg/kg PAT levels (Cunha et al., 2014).

2.4 | Alternaria (ALT)

The *Alternaria* species are generally saprophytic and produce an array of secondary metabolites; however, a minute proportion of mycotoxins are also identified (Noser et al., 2011). They include AOH, AME, TeA, tentoxin (TEN), and ALT, which are characterized and documented from cereals, nuts, citrus fruits like apples and oranges (Ji et al., 2022a). Amongst fruits, tomatoes are the most contaminated by ALT toxins, and their products, like ketchup, sauces, and purees, are frequently analyzed for the quantification of ALT (Zhao et al., 2015). Alarming values are reported by a recent study in China on the presence of ALT toxins in tomatoes and tomato products. According to the study, more than 50% of the samples were contaminated with one or more combinations of ALT mycotoxin. Canned and dry tomato powders, sauces, ketchup, juices, and dried tomato had 100, 89.6, 82.1, 46.7, and 20% toxins, respectively. Surprisingly, the fresh tomatoes had no detachable mycotoxins. Amongst the various forms of ALT, TeA was the leading contributor, with an occurrence level of up to 7985 µg/kg (Ji et al., 2022b). It can be inferred that production and processing strategies are contributing factors to the inoculation of the mold in the fruit product. Therefore, it is considered a major red flag and a potential health concern, particularly in a population consuming a large proportion of tomatoes. A risk assessment analysis performed among the European population stated that threshold levels for AOH and AME exceeded the permissible limits of 2.5 ng/kg (BW/D) in the vegan cohorts of toddlers (Arcella et al., 2016). The study posed a wide awareness of the effects of ALT in the vulnerable population, where the risk is expected to be higher as their gastrointestinal efficacy of breaking-down the toxins is underdeveloped. As a result, they exhibit a lower ability for

chemical breakdown, which increases the accumulation of toxins in the internal organs leading to neurotoxic, endocrine disturbance, and toxic immunological effects up to 4 years old (Huybrechts et al., 2011).

Moreover, the fact is of much relevance that AOH produces androgenic effects, and its effects would be much more drastic in the case of children (Stypuła-Trębas et al., 2017). Infestation of the *Alternaria* species in pome fruits was reported in Italy. The mycotoxicological profile confirmed the presence of several morphotypes of *A. alternata* and *A. arborescens* causative of heart rot disease in the fruit. Tea was commonly found in all the samples; however, other forms, such as AOH, alternariol monomethyl ether, ALT, and TeN, were also detected (Aloi et al., 2021). A similar study underlined the probable risks of consuming European pear (*Pyrus communis* L.) with black/brown spots caused by *Stemphylium* and *Alternaria* species. The toxicological profile indicated the presence of 89.1% of TeA and AOH, 80% of altertoxin, and 50% of ALT. Further, the study highlighted the synergistic presence of ALT toxins exceeding 7.58×10^6 ng/kg in the analyzed fruit samples (Prencipe et al., 2022). It is significant to understand that the growth/mass of *Alternaria* sp. is not proportional to the production of the ALT toxins. In other words, it is proven that in artificially inoculated *Alternaria* sp. in yellow peach fruits under controlled conditions, AME, AOH, and TeA are detected in the rotten areas of the fruit. At the same time, TeA was also detected in the unrotten fleshy tissue of the fruit (Meng et al., 2021). *Alternaria* species also infect sweet cherries. In particular, TeA and TEN are present in 50% with a quantification range between 0.002 and 0.066 $\mu\text{g}/\text{kg}$ (Myresiotis et al., 2015). The infestation of the *A. tenuissima* species group was reported in blueberry fruits. However, another little cluster of molds, such as *home* and *Penicillium* sp, coexisted. The level of pathogenicity varied from moderate to high. The percentage of AOH, AME, and TA was 97% (0.14–119.18 mg/kg), 95% (1.23–901.74 mg/kg), 65% (0.13–2778 mg/kg), respectively (Greco et al., 2012). The variation in abiotic conditions influences the production of different metabolites *Alternaria* sp. In accordance with this fact, the presence of AOH, AME, and TEN was assessed in strawberry samples maintained at different temperature setups. It was evident that the high-temperature limit ($22 \pm 2^\circ\text{C}$) induced a maximum level of AOH ranging between 26 and 752 ng/g, while AME concentration ranged from 11 to 137 ng/g, TEN was completely undetectable in the samples (Juan et al., 2016)

2.5 | OTA and other mycotoxins

The OTA is one of the most detrimental and stringently monitored mycotoxins worldwide. The molds of the genus *Aspergillus* and *Penicillium* (Ozer et al., 2012) were identified to produce large amounts of mycotoxins, particularly OTA and fumonisins (FUM) (Pitt and Hocking, 2009). OTA producers are nine species of fungi of the *Aspergillus* and *Penicillium* group. They correspond to the Circumdati cluster, which includes *A. ochraceus*, *A. westerdijkiae*, and *A. steynii*. On the other hand, the *Aspergillus* section Nigri group includes *A. carbonarius*, *A. niger*, *A. lacticoffeatus*, *A. sclerotioniger*, and *A. tubingensis* (Malir et al., 2016). Other significant OTA producers of *Penicillium* genera are *P. cyclopean*,

P. viridicatum, and *P. chrysogenum*, classified under *P. viridicatum*. However, the widely known producers are *P. verrucosum* and *P. nordicum* (Álvarez et al., 2020). Most of the *Aspergillus* fungi infest meat and meat products. Indeed fruits and vegetables are infected by *Aspergillus* and *Alternaria* during preharvest, while that *Fusarium* and *Penicillium* at the postharvest processes (Nan et al., 2022). Amongst the wide members in the genus, *A. nigri* was a potent source of OTA, especially in grapes, due to which the contamination is identified in grape juices, dried grapes, and wine (Pantelides et al., 2017). A study reported that the *Aspergillus* cluster comprising *A. carbonarius*, *A. luchuensis*, *A. niger*, *A. tubingensis*, and *A. welwitschiae* was identified in the grapes. However, the predominant colonizer in dried grapes was *A. tubingensis* (Merlera et al., 2015). Higher levels of OTA contamination of 75% were recorded in the raisin samples recently. However, in earlier studies, the levels of OTA ranged at minimum limits between 20% (Asghar et al., 2016). Similarly, another study reported that the OTA content in the dried vine fruit ranged between 0.8 and 10.6 $\mu\text{g}/\text{kg}$. However, the tolerable concentration posed by the European Commission was 10 $\mu\text{g}/\text{kg}$ (Mikušová et al., 2020). Further, variation in the levels of OTA in dried vine fruits varied across the regions. Abiotic factors like humidity, weather, temperature, harvesting time, and handling methods play an influential role in the OTA production of fruits (Castaldo et al., 2019). Amongst the wide variety of fruits, figs carry a high risk of being affected by mycotoxigenic species. The most common ones include *Aspergillus nigri*, *Fusarium* sp., *A. flavi*, and *Penicillium* sp, while other genera such as *Acremonium*, *Byssosclamyces*, *Cladosporium*, *Trichoderma*, *Mucor*, and *Scopulariopsis* were reported earlier (Isman & Biyik, 2009). The OTA has also been widely known as a noxious mycotoxin metabolite in extensive food products, including grains, coffee, dried fruits, nuts, spices, beer, fruit juices, wine, grapes, and its related products. The toxicity of OTA is fatal as it causes genotoxicity, hepatotoxicity, immunotoxicity, teratogenicity, and neurotoxicity (Ghali et al., 2009) and is also a precursor for the formation of urinary tumors associated with the Balkan endemic nephropathy. The tolerable threshold quantity of OTA for weekly intake is stipulated to be 120 ng/kg/body weight (Ostry et al., 2015). However, the maximal permissible limit is 10.0 ng/g in the case of the dried vine and other fruits. The incidence of OTA analyzed in the dried figs, apricots, dates, and raisins in Iran were 10.4, 6.7, 10, and 44.7%, respectively. The concentration of OTA in figs and raisins was higher than the maximum tolerable limits posed by the EU (Shakerian et al., 2013). *F. verticillioides* and *F. proliferatum* (Fotso et al., 2002) and a few species of *Aspergillus* sp, such as *A. niger* and *A. welwitschiae*, produce FUM such as FB1, FB2, and FB3 (Bhat et al., 2010), which are reported to be carcinogenic and causative for oesophageal cancer China and South Africa (Munkvold, 2017). It is significant to note that the studies on the wide sampling of occurrence of OTA in fruits, fruit products, raisins, and grapes occupy higher infection rates (Nikolchina and Rodrigues et al., 2021). In other cases, other fungal infection, such as powdery mildew caused by *Erysiphe necator*, causes extensive bruises and cracks on the fruit surfaces, which in turn provides space for the growth of *Aspergillus*, which effectively produces and accumulates FB2 and OTA in infected berries (Cozzi et al., 2013). *Fusarium* species also produce ZEA and are a potential contaminant

traced in fruits and fruit juice (Zinedine et al., 2007). *F. verticillioides* have been reported to synthesize ZEA at a concentration of 0.8–1 mg/g in a ripe banana during harvest. Furthermore, ZEA was also found to occur in tomatoes, avocados, melons, and bananas at 0.05, 3.5, 0.2, and 0.05 mg/40 g, respectively (Kalagatur et al., 2018). Research progress on the studies of ZEA unveiled that few others belonging to *Fusarium*, such as *F. chlamydosporum*, *F. circinatum*, *F. semitectum*, *F. solani*, *F. thapsinum*, and *F. proliferatum* produced ZEA at high levels up to 0.912 µg/ml in laboratory conditions. ZEA has been assessed to be a potent ROS influencer (Gao et al., 2013), a harmful genotoxic and carcinogenic substance and classified under Group 3 carcinogens (IARC, 1993). The ZEA was identified in food and beverages consumed by toddlers in a diet study conducted in the Netherlands. ZEA and other mycotoxins were quantified with levels below the postulated permissible limits. However, chronic exposure certainly leads to critical health hazards (Pustjens et al., 2022)

3 | IDENTIFICATION AND QUANTIFICATION OF MYCOTOXINS

The inconsistency in the identification and quantification of the masking mycotoxins poses a serious challenge in the food processing and preservation sectors. Several chromatographic techniques have been employed to detect mycotoxins that occur individually or in combination described in Table 1. Analytical protocols are employed for rapid and accurate quantification of mycotoxins. The commonly adopted methods include liquid chromatography (LC) with customized detectors like MS, DAD, and FLD (Khaneghah et al., 2019). However, gas chromatography (GC) was extensively used to quantify mycotoxins, and HPLC–MS/MS occupied the epitome due to high-end sensitivity and accuracy. Furthermore, customized approaches like HPLC–FLD, HPLC–DAD, and LC–PDA also have been used widely (Zhu et al., 2016). On the other hand, GC with flame ionization detection (GC–FID) and GC with tandem mass spectrometry (GC–MS/MS) (Mahmoud et al., 2018) has been the choice among the Instrumental based protocols, in addition to immunoaffinity column is also adopted for high discrimination and efficacy for identifying and quantifying masked mycotoxins (Kiszkiel-Taudul, 2021). Due to the high complexity in structures and the chemical moiety of the mycotoxins, it is challenging to choose a single technique to detect them. However, a combinational approach that offers flexible, cost-effective, sensitive, routine, broad-based, and accurate solutions would be the need of the hour. Based on the requirements, several customized protocols have been designed and practically executed to analyze even feeble quantities of mycotoxins in the food and fruit matrices. The fundamental rules for assessing toxins are pretreatment, extraction, separation, and quantification. Solid phase extraction (SPE) has been extensively used estimation of ALT, FUM, PAT, and trichothecenes present in apple juice and fried figs using specific phase columns such as C-18-RP, SAX, silica gel SPE column and C-18-RP respectively. Similarly, silica gel precoated G-25 HR TLC plates are commonly used to detect CIT, trichothecenes, and OTA in apples, pears, and grapes, respectively (Abrunhosa et al., 2016).

HPLC and GC–MS are generally employed for the detection of common mycotoxins. However, integrated analytical approaches such as reversed-phase HPLC, microemulsion electrokinetic UV–HPLC, ultra HPLC–MS, HPLC compared with ELISA and TLC, GC–MS combined with the electronic nose, and LC–MS/MS (Yang et al., 2014) are carried out successfully. In the case of fruit juices, capillary electrophoresis has been comparatively effective. In support of the above clause, CE coupled with fluorescence-based detection reported the presence of PAT in apple juice (Murillo-Arbizu et al., 2010). Other determination methods like fluorimetric, colorimetric, aptasensors, ELISA, nanosensors, and molecular-based methods like PCR are also employed (Lancova et al., 2011). Figure 2 represents an outline of several analytical techniques adopted in mycotoxin identification.

4 | DETOXIFICATION OF MYCOTOXINS IN FRUITS AND FRUIT PRODUCTS

The PAT and OTA have been widely reported for toxicity in fruit and fruit products worldwide Gonçalves et al., 2019. However, other toxins like AF B1, B2, G1, and G2 (AFB1, AFB2, AG1, AFG2), FUM B1, B2, and B3 (FB1, FB2, FB3); DON and other trichothecenes; ZEN and ergot alkaloids (EAs) are present in negligible quantities. Mycotoxin contamination causes a significant impact on import and export, leading to economic drip. Further, they pose a critical health hazard to the consuming cohorts. Figure 3 demonstrates the health hazards of consuming mycotoxin-contaminated fruit and fruit products. Generally, fruits are rich in natural acids like citric, malic, and tartaric acids, which infuse the alkaline pH, favoring the growth of these toxigenic fungi through soft tissues and contaminating the commodities. Decontamination of these mycotoxins generally relies on three methods, physical, chemical, and biological protocols for removing or degrading the toxin levels in fruits and their products. The widely adopted physical methods include manual sorting, milling, dehulling, cleaning, usage of inorganic binders, and other combinational approaches. However, these can be adopted in cereals, grains, and dry samples, but it is even more challenging for fruits and fruit production (Manbolu et al., 2018). The OTA and PAT demonstrated high resistance against thermal treatments like pasteurization and distillation, which were experimentally proven to have unaffected toxicity levels in wine (Quintela et al., 2013). On the other hand, nonthermal treatments such as ultrafiltration, micropore membrane filtration, pulsed light (PL), and ionizing radiations like UV and hydrostatic pressure methods have been found impactful in the treatment of apple juices (Barreira et al., 2010). A concentration of 1.0 mg/ml of PAT in apple juice samples showed a significant reduction of up to 90% after the UV treatment (Zhu et al., 2016). However, these treatments can sometimes alter the fruit juices' sensorial attributes (Assatarakul et al., 2012). On a large-scale industrial setup, other nonthermal approaches like PL and high hydrostatic pressure processing (HPP) have been impactful in treating contaminated fruit purees and juices. Notably, PAT concentration in apple juice samples was 129 mg/L; post-PL treatment, a 46% reduction in the PAT levels was noted (Funes et al., 2013). Similarly, a mixed juice blend

TABLE 1 Detection of mycotoxins in fruit and fruit-based products

Name of the toxin	Causative species	Substrate	Technical adopted	limits of detection (LOD)	Reference
Patulin (PAT) Citrinin (CTN)	<i>Penicillium</i> and <i>Aspergillus</i>	Pome fruits, such as apples and pears	HPLC	0.006 µg/g 0.001 µg/g	Sadhasivam et al., 2021
Citrinin (CTN)	<i>Penicillium</i> and <i>Aspergillus</i>	Apples	HPLC	320–920 µg/kg	EFSA, 2012
		Apples, cherry, black currant		50–240 µg/kg	
		Tomato juice		0.2 µg/kg	
		Fig		60 µg/kg	
		Pears		50 µg/kg	
Citrinin (CTN)	<i>Aspergillus</i> , <i>Penicillium</i> , and <i>Monascus</i> .	Commercial Beers	TLC	6 µg/kg	Odhav & Naicker, 2002
		Dried grape	HPLC-FD	5.56 µg/kg	Oztas et al., 2020
		Grape	USAE-DLLME-HPLC- FLD	0.16 µg/kg	Oztas et al., 2020
		Orange	UPLC-MS/MS	40.3 µg/kg	Wang et al., 2016
		Sweet cherries	UPLC-MS/MS	2.2–7.9 µg/kg	
		Tomato	UPLC-MS/MS	1.1–8.4 µg/kg	
Patulin (PAT)	NM	Strawberry	UHPLC-ESI-MS/MS	2.17–636.05 µg/kg	Sadok et al., 2023
		Raspberry		41.8–695.86 µg/kg	
		Redcurrant		69.3–599.83 µg/kg	
		Sour cherry		53.8–105.73 µg/kg	
AlternariaToxin (ALT)	NM	Jujube	Pretreatment QuEChERS and UPLC-IMS/QTOF MS	22.89–7830.19 µg/kg	Fan et al., 2022
		Melon		0–175.92 µg/kg	
		Grape		0–112.97 µg/kg	
		Pear		0–90.97 µg/kg	
Aflatoxins	NM	Jujube	Pretreatment QuEChERS and UPLC-IMS/QTOF MS	0–112.55 µg/kg	Fan et al., 2022
AFG1		Jujube		0–43.66 µg/kg	
AFG2		Melon		0–23.74 and 0–30.3 µg/kg	
AFB2		Grape		1.06–35.67 µg/kg	
AFB2		Pear			

(Continues)

TABLE 1 (Continued)

Name of the toxin	Causative species	Substrate	Technical adopted	limits of detection (LOD)	Reference
Trichothecene Beauverin (BEA)	NM	Jujube Pear Jujube Grape		0–345.34 µg/kg 0–22.9 µg/kg 0–257.85 µg/kg 15.60 µg/kg	Fan et al., 2022
Patulin (PAT)		Jujube		0–257.85 µg/kg	Fan et al., 2022
Patulin (PAT)	Apple and pear products	Fapas, cloudy apple juice	LC–QTOF–MS	5.22–13.42 µg/L	Duncan et al., 2022
Patulin (PAT)	Formula based foods	Fapas, clear apple juice		22.2–57.0 µg/L	
Patulin (PAT)	Formula based foods	Apples and infant formula	HPLC–FL	2 µg/kg	Pokrzywa & Surma, 2022
Alternaria (ATs) AME AOH TeA	Apple juice Alternaria sp	Yellow peach (<i>Amygdalus persica</i>)	UHPLC–MS/MS	5.1–14.7 µg/kg 251.3 µg/kg 74.2 µg/kg 15,819.2 µg/kg	Meng et al., 2021
Alternaria (ATs)	NM	Mixed fruit models	UPLC–MS/MS with QuEChERS	1.32–54.89 µg/kg	Xing et al., 2021
Multimycotoxin OTA Zearalenone	NM	Raisins, plums, figs, and cranberries	LC–MS/MS	1–100 ng/g 10–1000 ng/g	Zhang et al., 2022
Patulin (PAT) Ochratoxin A (OTA)	Fruit juice samples	Mixed fruit	D μ SPE–HPLC–MS/MS	39.6 and 131.7 ng/L 24.8 and 82.6 ng/L	Mohebbi et al., 2022
Ochratoxin A (OTA)	<i>A. niger</i> , <i>A. tubingensis</i> and <i>A. flavus</i>	Date fruit	HPLC with fluorescence detection	0.75 µg/kg	Nikolchina & Rodrigues, 2021
Patulin (PAT)	NM	Fruits and fruit-based products	HCR and hemin/G-quadruplex DNAzyme	1.23–16.4 µg/kg	Lu et al., 2022
Patulin (PAT)	NM	Dried fruits	ELISA	4102.0 µg/kg	Przybylska et al., 2021
Aflatoxin B1 (AFB1)	<i>Aspergillus niger</i> , <i>A. flavus</i> and <i>Fusarium</i> sp	Dry dates	HP–TLC	0.000303–0.03636 mg/kg	Awan et al., 2021

*NM, not mentioned.

TABLE 2 Biological degradation of major toxins by enzymes originated from microorganisms

Name of the toxin	Enzyme	Microorganism	Activity	Reference
Aflatoxin (AF)	AF-detoxifzyme (ADTZ)	<i>Armillariella tabescens</i> (E-20)	Detoxification of difuran ring of aflatoxin B1 (AFB1)	Wu et al., 2015
	Oxidoreductase BADE	<i>Bacillus shackletonii</i>	Activation of Cu ²⁺ and inhibition Mn ²⁺	Xu et al., 2017
	AF oxidase, extracellular enzyme	<i>Pleurotus ostreatus</i> and <i>A. tabescens</i>	AF-degradation activity	Motomura et al., 2003
	F420H2-dependent reductase	<i>Mycobacteria smegmatis</i>	Catalyses AF degradation activation of Mg ²⁺ and inhibition Li ²⁺	Taylor et al., 2010
	Myxobacteria AF degradation enzyme (MADE)	<i>Myxococcus fulvus</i>		
Ochratoxin (OTA)	MnP	<i>Phanerochaete sordida</i> YK-624	Catalyzes AFB1 detoxification and hydrolysis	Gonçalves et al., 2019
	Multienzyme	<i>A. tabescens</i>	Degradation of AFB1	
	Carboxypeptidase A Chymotrypsin Protease A Pancreatin	–	Hydrolysis	Abrunhosa et al., 2006
	Lipase	<i>A. niger</i>	Hydrolyzes OTA into OT α	
	Multienzymes	Black yeast <i>Exophiala spinifera</i>		Heinl et al., 2011
Deoxynivalenol (DEN)	Esterase FUMzyme	<i>Komagataella pastoris</i>		EFSA et al., 2014
	Epoxides	<i>Eubacterium</i> BBSH 797	Degradation and cleavage of epoxy ring	Moss et al., 2004
	Enzymes encoded by genes TRI101 or TRI201	<i>Fusarium</i> genus	Detoxification of DON	Khatibi et al., 2011
	Multienzymes	<i>Agrobacterium</i> - <i>Rhizobium</i> strain E3-39	Detoxification of DON	
	Enzymes of Tri101 gene	<i>Fusarium sporotrichioides</i> <i>Fusarium graminearum</i>	Deepoxidation of DON	
Zearalenone (ZEN)	Trichothecene-3-O-acetyltransferases	<i>Fusarium</i> species		
	Multienzymes	<i>Clonostachys rosea</i>	Conversion into nonestrogenic	Alberts et al., 2009
	Lactonase ZENC	<i>Neurospora crassa</i>	Inhibition: Zn ²⁺	Bi et al., 2018
	Laccases	Several fungal species	Hydrolysis	
	Lactonohydrolase enzyme	<i>Clonostachys rosea</i>	ZEN to a less estrogenic compound	Takahashi-Ando et al., 2005
Patulin (PAT)	Multienzymes	<i>A. niger</i> strain FS10		
	Multienzymes	Marine yeast <i>Rhodospiridium paludigenum</i>	Biodegradation under in vitro conditions	Zhu et al., 2015
	Multienzymes	<i>Enterococcus faecium</i> M74 <i>Enterococcus faecium</i> EF031		Topcu et al., 2010
	Multienzymes	<i>Saccharomyces cerevisiae</i>		Yue et al., 2011
		<i>Metschnikowia pulcherrima</i> (Yeast)		Reddy et al., 2011
	<i>Meyerozyma guilliermondii</i>	Reduction under alkaline conditions	Chen et al., 2017	

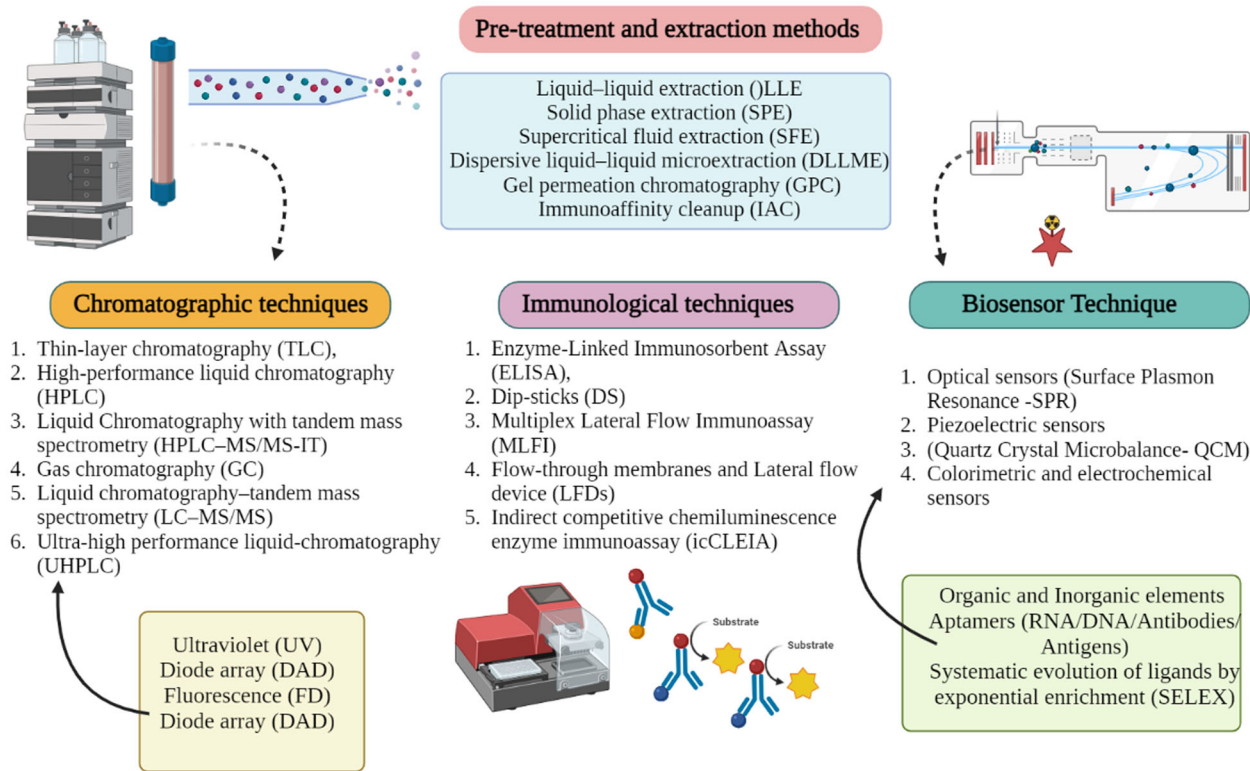


FIGURE 2 Schematic representation of analytical methods applied for the detection of mycotoxins in food matrices

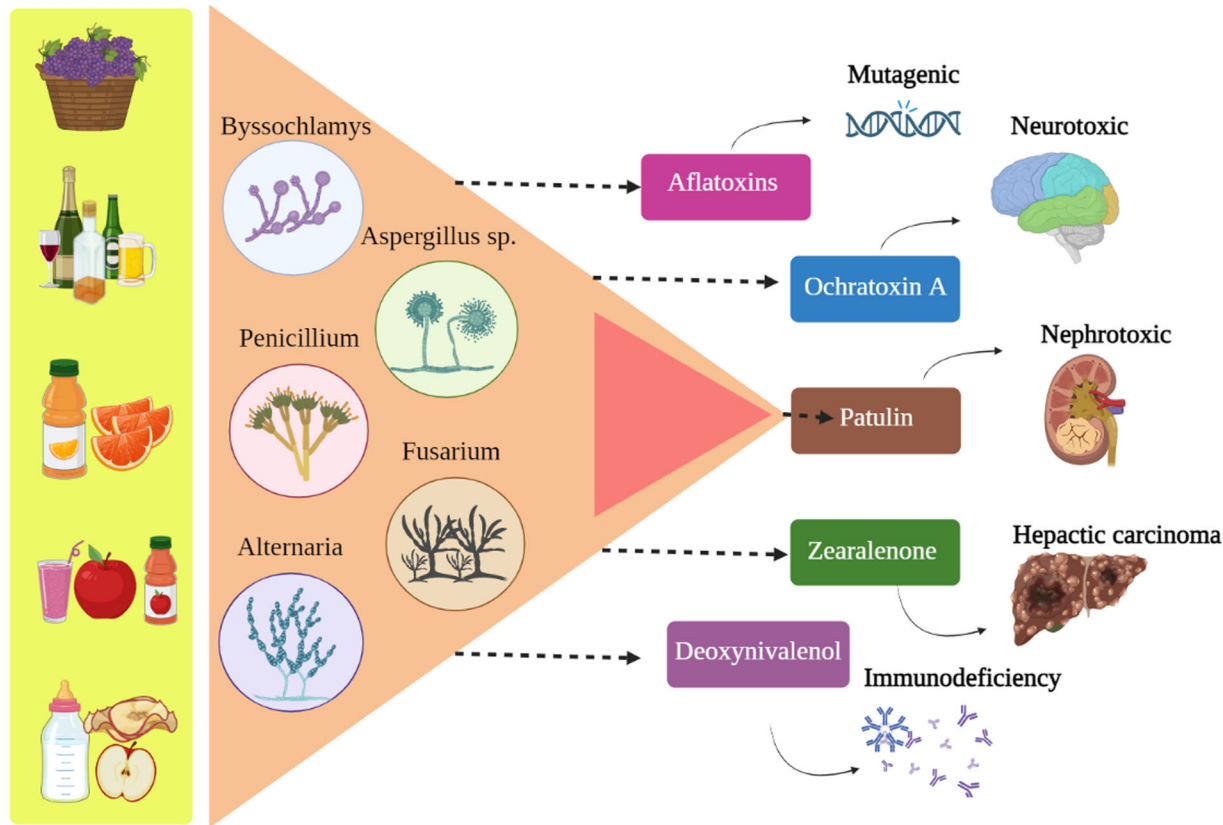


FIGURE 3 Schematic representation of health hazards caused by predominant mycotoxins in fruits and its products

comprising apple, pineapple, and mint presented a PAT level of 0.2 mg/L, the impactful reduction was noted on the levels of PAT up to 6 and 8 adopting the HPP of 400 and 600 MPa for 180 s, respectively (Hao et al., 2016). Apart from these physical treatments, one of the best approaches is the principle of adsorption using fining agents like charcoal (Kadalkal & Nas, 2002), bentonite (Bissessur et al., 2001), silica gel, and potassium caseinate (Abrunhosa et al., 2016) which had demonstrated a reduction of mycotoxin level up to 100, 77, 82, and 85% respectively. Biological methods include adopting the aid of microorganisms like yeast, *Alicyclobacillus acidoterrestris* (ATT92 and ATT96), *Saccharomyces cerevisiae* strains, *Lactobacilli* such as *Pediococcus parvulus*, *Candida intermedia* has been widely reported (Gonçalves et al., 2019). Lactic acid bacteria (LAB) are a rich source of anti-microbial compounds. They are employed in biopreservation and are a natural tool to inhibit fungal growth in feeds and foodstuffs (Bangar et al., 2022). Kiwi juice with a PAT level of 200 mg/L was treated with *Candida tropicalis*, and *A. acidocaldarius* demonstrated a degradation percent of 75 and 32, respectively (Luo et al., 2016). Degradation of *S. cerevisiae* strains has been extensively used in the reduction of OTA levels in model wine up to 2–82% (Petruzzi et al., 2014a), red wine up to 1–71% (Sun et al., 2017), white wine up to 6.5% (Espejo et al., 2016). Decontamination of PAT and OTA through chemical treatment is a challenging task. Several factors like stability, magnification, and interference in the composition of food products are potential factors for choosing chemical decontaminants. Conventional treatments like ammoniation and potassium permanganate solution are generally used; however, recent studies have demonstrated a significant reduction of ascorbic acid, B vitamins, and vitamin C degraded 68, and 70%, respectively; Yazici & Velioglu, 2002. The choice of biodegradation by enzymes is an effective and eco-friendly method of mycotoxin degradation, and it also supports maintaining the quality of fruit and its products. The enzymes responsible for the degradation of AFs are laccases, peroxidases, oxidases, and reductases. Table 2 represents various enzymes originating from microorganisms for the degradation of mycotoxins.

5 | FUTURE PERSPECTIVES AND CONCLUSION

Mycotoxin contamination is an unavoidable peril in fruits and processed fruit products. It has gained increasing concern for safeguarding human health due to its critical acute and chronic health hazards and impacts, even at low levels. Based on the review, the predominant occurrence of PAT, OTA, and AFs was recorded in apples, grapes, and figs, respectively (Sakuda & Kimura, 2010). Preharvest, postharvest, processing techniques, and co-occurrence of one or more toxins require more focus and concern from a toxicological point of view. In particular, children, adolescents, and youngsters consume more fruit-based beverages, wines, and other fruit-based products and are the most vulnerable group to severe health hazards. Application and development of inter-disciplinary approaches like DNA/RNA aptamers-based sensors, nano-based sensors, quantum dots, quartz crystal microbalance, electrochemical biosensors, Ag–Ab-based sensors, surface plasmon resonance sensors (Pushparaj et al., 2022) have

been effectively employed exclusively for detection of mycotoxins. Very recently, knowledge of the microbial consortium, application of metabolomics, and tri-trophic interactions in improving postharvest storage and fruit quality has significantly influenced mitigation strategies. Similarly, another interesting domain capable of mitigating the mycotoxins is through mirroring the conventional biocontrol approach, wherein nuances of host–fungal interactions are manipulated by artificially induced biofilms or host–microbiome manipulation methods (Bartholomew et al., 2021). Apart from detection and quantification strategies, following a standard protocol for agricultural practices and culture methods, periodical application of biocontrol agents, fungicides, and insecticides, adoption, and monitoring of integrated management system throughout the cycle of seeding, harvest, postharvest, processing, and storage is the pressing need of the hour. Studies on the efficacy of LAB strains *Pediococcus* sp. in decontamination in food matrices have been successful (Park et al., 2022). However, potential improvisation is needed to make a tangible approach in practice. Certainly, with the rapid advancements in biotechnology, metabolomics, and genetic engineering, developing improved crop varieties with resistance to mycotoxins can also help to eliminate them from exploiting the food web.

AUTHOR CONTRIBUTION

Conceptualization: B. B. and W. C-L. **Writing—original draft preparation:** K. P. and B. B. **Selected bibliographic sources:** A. M., K. P., M. P., and A. M. K. **Coordinated the working group:** B. B. **Writing—review and editing:** A. M. K., B. B., W-C. L., and A. M. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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