



## Mycotoxins in Fruit and Fruit Products

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The term “mycotoxin” is generally associated with secondary metabolic toxic products of filamentous fungi to which humans and animals can be exposed, most often through ingestion. This exposure can result in a range of toxicities (acute to chronic), and a spectrum of effects (mild to severe) including carcinogenicity and death. The history of mycotoxicoses has been long and dramatic, including a cereal-associated outbreak of ergot contamination that is thought to have catalyzed the unfortunate events known as the Salem Witch Trials. While many significant mycotoxins are associated with grain-based food products, fruit and fruit products can also be affected. Patulin, a mycotoxin produced by many *Penicillium* spp., is a known hazard in the apple juice industry where its levels are under regulatory control (no more than 50 ppb in apple juice). Orange juice and fermented fruit beverages such as wine are also susceptible to mycotoxin contamination from *Fusarium* and *Aspergillus* spp., respectively. Other fruits support the growth of mycotoxin-producing fungi, although specific conditions that lead to the production of mycotoxins are not well-elucidated. Inconsistencies in reports and lack of survey data on the prevalence and level of mycotoxins in fruit products makes the risk assessment of mycotoxins in fruit products challenging.

The term mycotoxin is derived from the Greek word “mycos” meaning mold, and the Latin word “toxicum” meaning poison. Harmful to humans and animals that may ingest them, mycotoxins are the low molecular weight secondary metabolites of various fungi. Mycotoxins are considered secondary metabolites because they are not needed for fungal growth, and are produced by primary metabolic processes (Bellí, 2006). Although they have been studied since the late 1960s, the exact function of mycotoxins remains a mystery. It is hypothesized that mycotoxins act as a defense mechanism, protecting the fungus from plants, animals and other competing fungi (Smith and Moss, 1985). The infectious dose of mycotoxins necessary to produce adverse health effects varies between fungi and the infected animal or human. Toxicological effects on humans and animals can be either acute or chronic. Acute toxicity is the rapid onset of an adverse effect from a single exposure, whereas chronic toxicity is a slow or delayed onset and is typically due to multiple or long term exposure. Most often, ingestion of mycotoxins and the toxicity they confer to humans is through chronic exposure over long periods. Chronic exposure, being the primary mode of affliction, creates difficulties in risk assessment and mitigation across populations, especially those in developing countries. The objective of this review is to highlight the major fungal species and the mycotoxins they produce that can potentially contaminate fruit and fruit products.

### Patulin

Patulin (4-hydroxy-4H furo [3, 2c] pyran-2[6H]-one) is a mycotoxin produced by several fungal species including *Penicillium* spp., *Aspergillus* spp., and *Byssoschlamys* spp. (Kurtzman and Blackburn, 2005). The most common causal agent of patulin is *P. expansum*, which has been demonstrated to contaminate fruit and fruit products as “blue mold rot.” However, the presence of

patulin-producing fungi does not ensure mycotoxin production (Moss, 2008). *P. expansum* is an organism of a ubiquitous nature within agricultural environments and is known to contaminate various food products. Fruit and fruit products that are susceptible to contamination from this microorganism include: apples, apricots, grapes, pears, peaches, sour cherry, black currant, orange, pineapple, strawberry, cranberry, and passion fruit (Moake et al., 2005). In particular, apples have been found to be contaminated with patulin concentrations ranging from 800–12,500  $\mu\text{g}\cdot\text{kg}^{-1}$  of rotten tissue (Reddy et al., 2010). Preharvest damaged fruit or those infected by other microorganisms are easily colonized by this pathogen in orchards or under postharvest conditions (Snowdon, 2001). The production of patulin within fruit and fruit products has been directly linked to multiple factors i.e. water activity, temperature, and pH. The optimum temperature for patulin production is within the range of 23 to 25 °C. Studies have suggested that lower storage temperatures reduce patulin production when compared to higher temperatures, but production still exists at temperatures as low as 0 °C (Hasan, 2000; Reddy et al., 2010). With regard to pH, patulin is relatively unstable at high pH, with instances of *Penicillium* spp. secreting organic acids to acidify, thus stabilizing the surrounding environment for patulin production (Tsao and Zhou, 2000). Not only is the toxin stable in acidic environments, it is also heat-stable, readily surviving thermal processing (Acar et al., 1998), and leaving low acid fruit juices especially susceptible to contamination. According to Spadaro et al. (2008), the high water solubility of patulin aides in its contamination of fruit juice concentrates. The susceptibility of alcoholic fruit beverages and vinegars to patulin contamination is minimal due to the fermentation step during their production (Gonzales et al., 2007). Although patulin is considered a group-3 carcinogen by the International Agency for Research on Cancer (IARC), there is no evidence of carcinogenicity in humans (IARC, 1987) but, reports have cited patulin as a teratogenic and genotoxic agent (Liu et al., 2003). The toxicity of patulin is

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believed to be associated with the binding of sulfhydryl groups, proteins, and amino acids within the plasma membrane (Fliege and Metzler, 2000). From animal studies, this mycotoxin has been noted to cause damage to respiratory and gastrointestinal systems (Mahfoud et al., 2002), enzymes, and DNA in human cells (Wichmann et al., 2002). Oxidative damage which yields a depletion of glutathione levels has also been attributed to the action of this toxin (Riley and Showker, 1991). Toxicity is dose-dependent with rapid gastrointestinal degradation after ingestion (Rychlik, 2003), and in the short term yields symptoms such as convulsions, dyspnea, pulmonary edema, and vomiting (World Health Organization, 1996). Long term toxicity has been shown to reduce immune system function and increased development of fibrosarcomas (Dickens and Jones, 1961). To this end, a provisional maximum tolerable daily intake (PMTDI) of  $0.4 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{body weight}$  has been established based on a “no observed effect level” (NOAEL) [Joint FAO/WHO Expert Committee on Food Additives (JEFCA), 1995]. Due to this guideline, within the European Union, patulin is regulated at levels of  $50 \mu\text{g}\cdot\text{kg}^{-1}$  in fruit juices and nectars,  $25 \mu\text{g}\cdot\text{kg}^{-1}$  in solid apple products, and  $10 \mu\text{g}\cdot\text{kg}^{-1}$  in apple-based products for young children and infants (EC, 2005). The U.S. Food and Drug Administration (USFDA) limits patulin to  $50 \mu\text{g}\cdot\text{L}^{-1}$  in single-strength, reconstituted apple juices, and fruit products (USFDA, 2004). Controlling patulin contamination of fruit juices and products is possible via sorting damaged and rotted fruit, using healthy fruit, sanitary storage bins/containers, activated charcoal filtration, irradiation, and addition of sulfur dioxide (Yun et al., 2008). With regard to trimming areas of fruit colonized by blue mold, recommendations are mixed. Some studies suggest this as a viable means of excluding the contamination source whereas others such as Laidou et al. (2001), noted that up to 25% of mycotoxins are detectable in the sound tissues around infected tissues in pears; and a 1–2 cm patulin diffusion from rotten apples (Marin et al., 2006a). Reducing the time that apples stored in controlled cold rooms are allowed to equilibrate (typically 2–4 d) and delivered to processors (another 3–7 d) can reduce the exponential spore density increase demonstrated under these operating conditions; lowering contamination probabilities (Amiri and Bompeix, 2005). These control methods in conjunction with the adoption of good manufacturing practices, adherence to moisture controls, following HACCP principles, and implementation of good quality assurance programs would greatly reduce patulin juice contamination incidences (Moake et al., 2005).

### Aflatoxin

Aflatoxins are secondary metabolites primarily caused by select isolates of *Aspergillus flavus*, *A. nomius*, and *A. parasiticus*; however, not all isolates are toxigenic. The ability to synthesize aflatoxins is strain-dependent, and occurs in the fungal hyphae, conidia, and sclerotia. Maximum mycotoxin levels are encountered when fungal mycelium reach an optimal level (Baird et al., 2006; Varma and Verma, 1987). The optimal conditions for *A. flavus* and *A. parasiticus* growth and toxin production are  $35^\circ\text{C}$  with  $0.95 a_w$  and  $33^\circ\text{C}$  with  $0.99 a_w$ , respectively. Temperatures below  $7.5^\circ\text{C}$  and above  $40^\circ\text{C}$  will inhibit the toxin production of both species. *Aspergillus* fungi are ubiquitous within the environment and are considered a saprophytic species, colonizing plant debris and deteriorating crops (Battilani et al., 2008). The major aflatoxins produced by these fungi are  $B_1$ ,  $B_2$ ,  $G_1$ , and  $G_2$ . Toxins  $B_1$  and  $G_1$  occur most frequently and in the largest quantities in fruit and their associated products. The order of acute

and chronic toxicity is  $B_1 > G_1 > B_2 > G_2$ , and is in direct relation to toxin structure, rendering higher potency via the cyclopentenone ring and the 8,9-double bond epoxidation of the B series, which contrasts with the six-membered lactone ring of the G series (Mclean and Dutton, 1995). Aflatoxin contamination has been known to affect peanuts, pistachios, almonds, hazelnuts, figs, dates, citrus, and raisins (Shundo et al., 2003). Studies have shown that aflatoxin contamination of individual figs has reached levels above  $600 \mu\text{g}\cdot\text{kg}^{-1}$  in 83% of figs infected by *A. parasiticus* and 38% from *A. flavus* colonization. Only figs contaminated by *A. parasiticus* contained both B and G toxins, while *A. flavus* contamination only yielded B toxins (Doster et al., 1996). Fig colonization by *Aspergillus* spp. is directly related to fruit age/maturity. Younger green figs are resistant to infection, whereas further ripened, softer figs have a decreased capacity for resistance. *A. flavus* has been noted as having the ability to penetrate the inner cavity of figs (Buchanan et al., 1975). Dates have also shown susceptibility to contamination from this mycotoxin after storage in facilities with elevated humidity levels. Aflatoxin  $B_1$  was detected in these samples ranging from 35 to  $11,610 \mu\text{g}\cdot\text{kg}^{-1}$ . Fresh and dried samples were found to be naturally devoid of aflatoxins when stored under proper conditions (Shenasi et al., 2002). With regard to citrus, aflatoxin production within oranges was found to be a consequence of initial infections that caused peel deterioration from other microorganisms. After infection, 69% of the aflatoxin was detected in the peel, 13% in the pulp, and at least 35% in the clear juice (Ragab et al., 1999). One unique characteristic of *A. parasiticus* and *A. flavus* as elucidated by Sharma et al. (1985) is the ability of these two fungi to produce ethylene during the early growth phase. Ethylene, a biologically active compound, has been found to inhibit aflatoxin production at 0.1–150 ppm. Toxin biosynthesis is thought to be controlled by an ethylene sensor within the fungus, with inhibition occurring at the transcription level (Roze et al., 2004). However, there are several factors that may alter its effects such as  $\text{CO}_2$  presence, substrate, temperature, and 1-methylcyclopropene, an ethylene-inhibiting compound. Aflatoxin  $B_1$  is recognized as a carcinogen, causing malignant tumors, and primarily targeting the liver. The International Agency for Research on Cancer classifies this toxin as a group I carcinogen and is thought to be the cause of human primary hepatocellular carcinoma (IARC, 2002). The DNA binding properties of aflatoxins is thought to be a key component in its carcinogenic and mutagenic activity, as well as having an association with its acute toxicity. The study of the teratogenic nature of aflatoxins has resulted in the demonstration of organ malformations in mammals. Chromosomal mutations and alterations comprise the basis of teratogenicity owing itself once again to the protein-binding activity of this mycotoxin (Stark, 2001). Due to the dangers associated with aflatoxins, the FDA has set action levels for products meant for human consumption at  $20 \mu\text{g}\cdot\text{kg}^{-1}$ , and  $0.5 \mu\text{g}\cdot\text{kg}^{-1}$  for milk (Dors et al., 2011). Due to the heat-stable nature of aflatoxins, decontamination within acceptable levels for fruit products can be challenging. The best means of controlling aflatoxins is by overall prevention through the use of good agricultural and manufacturing practices. It is recognized that even with these procedures in place, there will be instances where contamination occurs and has to be managed. Aflatoxins may be removed from contaminated fruit products via physical, chemical, or biological means. Due to the delicate balance within the matrix of fruit products not all of these methods are feasible or cost effective. One method in particular that has shown promise is the use of ozone for aflatoxin decontamina-

tion. Since being given GRAS (Generally Recognized As Safe) status by the U.S. FDA in 2001, ozone has proved successful for aflatoxin reduction in corn and other grains used for human consumption (Dors et al., 2011).

### Ochratoxin

Since being first documented by South African researchers in 1969, ochratoxin has been a common mycotoxin of study due to its contamination of cereals and their byproducts (Shotwell et al., 1969). After the natural susceptibility of grapes to *Aspergillus* and *Penicillium* spp. was identified, wine, dried grapes, and grape juice contamination by ochratoxin became an investigative focus. Ochratoxins A ( $C_{20}H_{18}O_6NCl$ ) and B ( $C_{20}H_{19}O_6N$ ) are both produced by *Aspergillus* strains, but ochratoxin A (OTA) is found to be produced with greater frequency and is the more toxic of the two (Abramson, 1997). The similar structure of one section of the OTA to the amino acid phenylalanine predicates its biochemical effects in humans. The inhibition of RNA and DNA synthesis has been observed as a mechanism of OTA; however, its overriding effect is the inhibition of protein synthesis. This inhibition is presumably due to the toxin outcompeting enzymes that also use phenylalanine as substrate (Aish et al., 2004). According to Varga et al., (1996) OTA is produced by the following *Aspergillus* strains: *A. ochraceus*, *A. alliaceus*, *A. sclerotiorum*, *A. sulphureus*, *A. albertensis*, and *A. auricomus* and *A. wentii*, and *Penicillium verrucosum*. OTA contamination by *P. verrucosum* is typically found in temperate climates, whereas OTA production by *Aspergillus* species is demonstrated primarily in tropical and subtropical areas. With regard to grape contamination, *A. carbonarius* and *A. niger* were pinpointed as being OTA producers on this crop with culture medium levels ranging from 2 to 24.5 ng·mL<sup>-1</sup> (Chulze et al., 2006). In field samples, OTA produced by *A. carbonarius* was found in levels up to 37.5 µg·g<sup>-1</sup>, frequently occurring in vineyards with Mediterranean climates. Further isolation of OTA from healthy berries proved that the toxin could also be produced within grapes still in the vineyard (Serra et al., 2006). During in vitro studies, *A. carbonarius* strains were found to grow optimally at 30 to 35 °C, with minimal growth at <15 °C, and no detectable concentrations of OTA at 35 °C. Temperature optimums for toxin production were observed between 15 and 20 °C. The optimum  $a_w$  for the consistent growth of this fungus was found to vary between 0.93 and 0.98 (Marin et al., 2006b). Factors that lead to fungal colonization and OTA production include: climatic conditions and latitude, contact of berries and mycotoxigenic fungi, grape cultivation practices, wounds or injuries that allow fungal penetration, and processing techniques including fermentation conditions (Blesa et al., 2006). It must also be noted that differences in OTA contamination among grape varieties was correlated most strongly with variations in climate and timing of fruit maturation more so than characteristics of the varieties (Leong et al., 2006). Grape susceptibility and colonization by *Aspergillus* spp. was evident as early as one month after berry setting, possibly because the surface of immature berries and UV exposure create an inhospitable environment for ochratoxigenic fungi. The highest levels of infection were observed during early veraison, ripening, and further berry maturation when fruit skins have softened and sugar content increases. Fungal growth rates were contingent upon the fungal isolate and species involved in the colonization, *A. carbonarius* was noted to be very invasive, penetrating intact berries, whereas *A. fumigatus* demonstrated more opportunistic characteristics, infecting the pulp of wounded grapes (Battilani et al., 2003). Varied colonization patterns such

as these undoubtedly affect the toxin concentrations from OTA producers. Grape contamination in the field has led to the subsequent contamination of grape products, i.e., grape juice and wine. Grape juice is often consumed by children, which provides greater justification for the investigation of production conditions that lead to potential OTA production. Studies have revealed OTA contamination at higher concentration levels in red grape juice than white, with ranges of 100–5300 ng·L<sup>-1</sup> and 71–1300 ng·L<sup>-1</sup>, respectively; which is thought to be caused by long time treatments to impart more color in the final juice product (Majerus et al., 2000). With regard to wine, OTA contamination has been detected in red, rosé, and white table wines. From surveys of studies performed in Europe, OTA contamination in red wines ranged from 388–2400 ng·L<sup>-1</sup>, 178–1200 ng·L<sup>-1</sup> in white wine and 123–2400 ng·L<sup>-1</sup> for rosé wines (Barkai-Golan, 2008). Contamination gradients such as they are thought to have a correlation with wine color. During the wine-making process, deeper hued wines are left in contact with the lees longer than lighter wines. Furthermore, lighter wines are clarified with bentonite or zeolite, which removes proteins that are simultaneously bound to OTA (Leong et al., 2007). In rare cases, airborne OTA infection has occurred, but poisoning is primarily due to the ingestion of contaminated foodstuffs (Richard et al., 1999). Identification of OTA exposure to humans in population studies has identified blood plasma concentrations within seemingly healthy people due to its long elimination half-life of around 35 d. After absorption is complete, OTA binds to human serum albumin rendering its concentrations quantifiable (Ilichev et al., 2002; Studer-Rohr et al., 1995). The toxicity of OTA is demonstrated via its nephrotoxic action, in which the kidney is its primary target organ, followed by the liver as a secondary site for tumor formation. It has also been cited as a factor in the disease Balkan Endemic Nephropathy and urinary tract tumor development (Creppy, 1999). The IRAC currently classifies OTA within Group 2B, a possible human carcinogen based on animal studies (IARC, 1993). Studies have shown that OTA exhibits hepatotoxic, teratogenic, genotoxic, and mutagenic effects, with immunosuppressive activity. When investigating the effect of red wine on OTA toxicity, it was noted that red wine limited the oxidative damage of OTA protecting against nephrotoxicity (Bertelli et al., 2005). Currently, the EU's maximum allowable OTA levels for wine and juice are 2 µg·L<sup>-1</sup>, and 10 ppb in dried grapes (EU commission, 2005), within the U.S. there is no stated maximum allowable limit for OTA contamination. According to Rousseau et al. (2005), and Gambuti et al. (2005), strategies for controlling of this mycotoxin postharvest in wine production include, but are not limited to: harvesting intact grapes to reduce leakage during transport, rapid transport to the winery, cool storage facilities, and overall sanitary wineries.

### Fumonisin

Typically not associated with fruit or fruit products, fumonisin ( $C_{34}H_{59}NO_{15}$ ) has recently become a topic of interest in the contamination of these agricultural products. Originally, fumonisin was thought to be a mycotoxin only produced by *Fusarium* molds, such as *Fusarium verticillioides* (Proctor et al., 2003). Recent studies have confirmed this to no longer be the case with confirmed production of fumonisin B<sub>2</sub> (FB<sub>2</sub>) by *Aspergillus niger* isolates (Frisvad et al., 2011). Other fumonisins such as B<sub>4</sub> and B<sub>6</sub> have also been produced by *A. niger* isolates, but at significantly lower concentrations. These fumonisin analogues differ structurally with hydroxyl groups located at different positions on the toxin molecule (Rheeder et al., 2002). FB<sub>2</sub> contamina-

tion caused by *A. niger* has been found in commodities such as cocoa beans, coffee beans, grapes, and dried fruit (Abrunhosa et al., 2011). This mycotoxin is a polyketide and has been noted to cause cancer and neural tube defects in several animal species. Inhibition of folic acid uptake via the folate receptor has also been demonstrated by fumonisins (Stevens and Tang, 1997). Furthermore, consumption of fumonisin-contaminated maize has been linked to esophageal cancer and neural tube defects in humans (Desjardins, 2006). Biochemical analyses of *F. verticillioides* identified the fumonisin gene cluster FUM, which is composed of 17 genes. This cluster of genes encodes a transcription factor, enzymes, and transport proteins (Proctor et al., 2006). Genome sequencing of *A. niger* uncovered a biosynthetic gene cluster, which includes orthologues of 10 genes also found in the *Fusarium FUM* cluster. The prevailing distinction between the two clusters is the *FUM* cluster of *Aspergillus* lacks the *FUM 2* gene which is directly related to the production of FB<sub>2</sub>, FB<sub>4</sub>, and FB<sub>6</sub>, in tandem with its *FUM 8* gene (Mansson et al., 2010). Three strains of *Aspergillus* that have been verified as producers of FB<sub>2</sub> through transcriptomic and metabolomic examination are ATCC 1015, NRRL 3, NRRL 567, and CBS 513.88. All three strains contain a fumonisin gene cluster similar to the cluster associated with fumonisin production by *Fusarium* spp. (Frisvad et al., 2011). Although maximum growth of *A. niger* is exhibited at 30 °C, the optimal in vitro temperature for FB<sub>2</sub> toxin production is 25 °C for most *A. niger* isolates. Mycotoxin production was also found to be at its peak at an *a<sub>w</sub>* range of 0.98–0.99. Studies have also shown that the addition of 5 % NaCl (*a<sub>w</sub>* = 0.97) or 20% sucrose (*a<sub>w</sub>* = 0.99) increases FB<sub>2</sub> production by *A. niger*, followed by a stagnation in production at higher sucrose concentrations (Mogensen et al., 2009). The average tolerable daily intake for FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub>, alone or combined within the European Union is 2 µg·kg<sup>-1</sup> bw·day<sup>-1</sup>. This dose is based on a no-observable-adverse-effect level (NOAEL) in the male rat kidney and incorporates a 100-fold safety factor (FAO/WHO, 2002). A ubiquitous species in vineyards, *A. niger* has produced detectable amounts of FB<sub>2</sub> in grapes, raisins, must, and wine. The risk of FB<sub>2</sub> is ever prevalent due to presence of *A. niger* aggregates which are present on grapes during the growing season and at higher levels than other black aspergilli (Logrieco, 2010). In wine, FB<sub>2</sub> contamination has ranged from 0.4 to 2.4 µg·L<sup>-1</sup>. According to Frisvad et al., (2011) strains of *A. niger* (83%) used in industrial applications were found to be producers of FB<sub>2</sub> when grown in conditions mimicking those of industrial citric acid production. Under these conditions there was no correlation found between fumonisin production capacity and growth rate, metabolism, secretion or any other biotechnical feature from industrial domestication of *A. niger*. Currently, there are no published validation methods for analysis of this mycotoxin in fermentation products, which also hinders any risk assessment activities.

The main concern for human health with regard to fruit contamination via mycotoxins within developed countries is presently focused on exposure to patulin, aflatoxin, ochratoxin A, and fumonisin. Due to the lessened feasibility of eliminating all potential hazards, fruit producers must rely on risk reduction rather than risk elimination (Zhao, 2005). To this end, fruit producers must place responsible culling and sorting efforts at the forefront of their operations. Removal of unhealthy fruit before they reach production lines is one way to reduce mycotoxin introduction into fruit products. The establishment and adherence to Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), and HACCP programs would also decrease the risks associated

with mycotoxin contaminated fruit products. Biological fungicide use would also contribute to decreases in mycotoxin occurrences as well. Although traditional pesticide use is an option, this may not be feasible for organic growers and those countries under intense pesticide regulations. Contamination by mycotoxins is recognized as an unavoidable risk, but careful and diligent planning and execution of daily procedures can substantially reduce this risk in fruit and fruit products.

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