MYCOTOXINS IN FRUITS: MICROBIOLOGY, OCCURRENCE, AND CHANGES DURING FRUIT PROCESSING

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I. INTRODUCTION

Mycotoxins are secondary metabolites of molds (primarily deuteromycetes), which are generally produced under optimum conditions at the end of the exponential growth phase. The term *mycotoxin* combines the terms "mykes," the Greek word for molds, and "toxicum," the Latin word for toxic or poisonous. Accordingly, mycotoxins occasionally also are defined as

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secondary metabolites of molds that exert toxic effects on animals and humans (1999). The physiological function of mycotoxins is not understood fully. Metabolic control mechanisms within the fungus, as well as defense mechanisms against other organisms, are discussed. Molds are characterized by a ruderal strategy of life, filamentous growth, intensive sporulation, primarily vegetative propagation, a parasexual life cycle, and a marked metabolic diversity, and they are distributed ubiquitously because the spores are disseminated with wind and water. About 300 fungal species are considered to fall into the group of molds that constitute an evolutionary relatively young branch of the Eumycota (Weidenbörner, 2000). Most species of the Eumycota have not been described, and conservative estimations are that at least 10^4 species occur on earth. Two unique secondary metabolites are assumed to be synthesized per species, and about 10% of the known secondary metabolites of fungi are mycotoxins, leading to the estimation of 2×10^3 mycotoxins, of which more than 300 have been characterized (Riley, 1998).

Only a few of these mycotoxins are regularly found in foods, with the predominant ones including aflatoxins, ochratoxin A, patulin, and different toxins produced by *Fusarium* species. The latter are only briefly discussed in this chapter, because their occurrence is mainly limited to grains and seeds (Parry *et al.*, 1995). In the last decade, another group of toxins produced by *Alternaria* species including alternariol, alternariol methyl ether, tenuazonic acid, altenuene, altertoxin I, and altertoxin II has attracted attention (Scott, 2001; Scott and Kanhere, 2001; Serdani *et al.*, 2002; Singh *et al.*, 2001; Tournas and Stack, 2001).

The history of mycotoxin research is closely related to research on fungal antibiotics; for example, patulin was first isolated from *Penicillium patulum* during the search for antibiotics in 1941 (Weidenbörner, 2001). After isolation of citrinin in 1931, it was first considered a highly effective antibiotic, but its toxic effects were discovered during antibiotic testing. Intensive research on aflatoxins and ochratoxin A started in the 1960s after some severe acute mycotoxicoses had occurred in domestic animals and humans in Japan, the Balkan region, the USSR, and England. Mycotoxins are toxic for vertebrates at low concentrations when ingested or inhaled (via spores) under natural conditions. Physicochemically, the mycotoxins are thermostable and in most cases aromatic and nonantigenic low-molecular-mass metabolites. Mycotoxins exert a diverse range of toxic effects because their chemical structures are very heterogeneous (Figure 1).

Apart from their acute and chronic toxicity, mycotoxins may possess carcinogenic, mutagenic, and teratogenic properties. They may act primarily on the liver (hepatotoxicity), kidney (nephrotoxicity), nervous (neurotoxicity), and immune systems (immunotoxicity or immunosuppression), on the uterus (uterotropism), and on the skin (dermatotoxicity), or they may act as

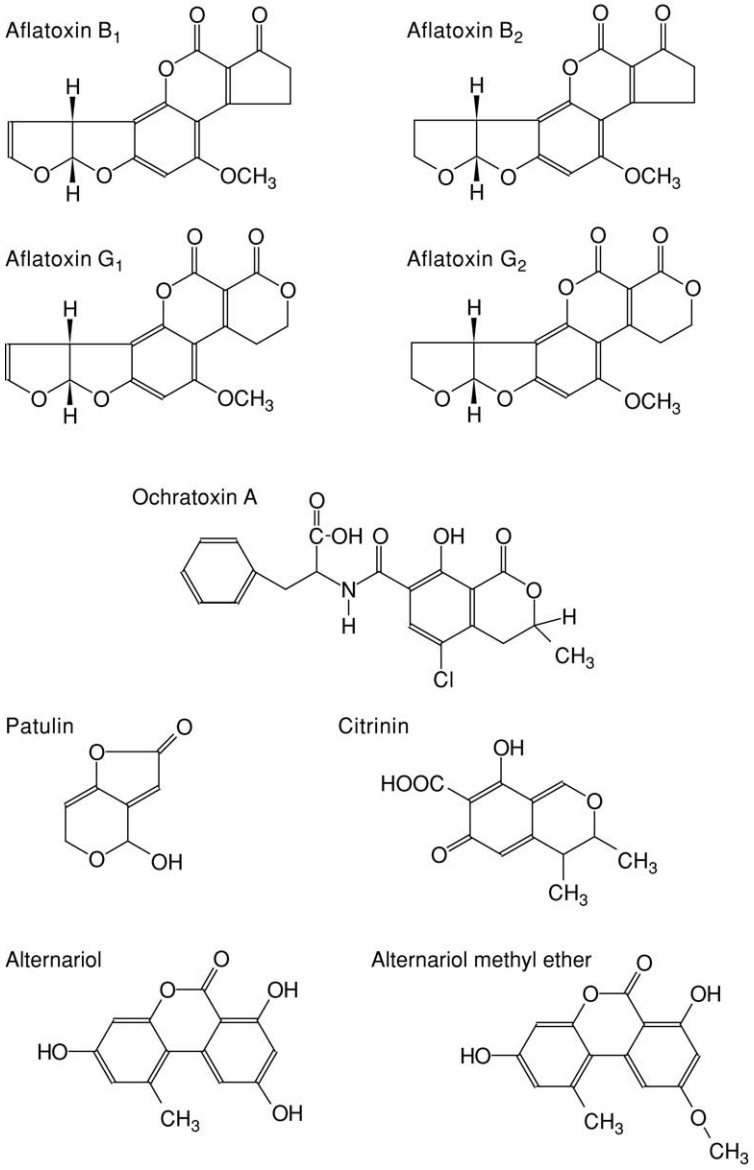

FIG. 1 Chemical structures of different mycotoxins.

general cytotoxins (Weidenbörner, 2000). Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic. The main target organ is the liver (Peraica *et al.*, 1999), and there is sufficient evidence for their hepatocarcinogenicity to humans (Dirheimer, 2000). The main target organ of ochratoxin A is the kidney. Apart from nephrotoxic effects, immunosuppressive, carcinogenic, and teratogenic effects are described. Ochratoxin A has been discussed as a main factor in the etiology of a kidney disease in Bulgaria, the former Yugoslavia, and Romania, which was first described in the 1950s and was named *Balkan endemic nephropathy*. Pfohl-Leszkowicz *et al.* (2002) reviewed scientific research on this topic and concluded that Balkan endemic nephropathy is of multifactorial origin, but the authors strongly believe that mycotoxins including ochratoxin A are among these factors. Toxicity of patulin is controversial. As summarized by Rychlik (2003), symptoms of acute and chronic toxicity have been observed in different feeding trials in rats in addition to teratogenic and immunotoxic effects. The author also cites different studies, in which orally administered patulin resulted in no signs of carcinogenicity, whereas subcutaneous injection produced sarcoma at the site of injection. Rychlik (2003) showed that patulin is rapidly degraded in humans in the blood before reaching other tissues outside the gastrointestinal tract.

Whereas endotoxins are restricted to the fungal mycelium, mycotoxins covered in this chapter exert their effects on other organisms as exotoxins. They diffuse into the environment and can be found in food and feed areas, which do not show any sign of mycelial growth. Therefore, the absence of molds does not guarantee freedom from mycotoxins, and conversely, the presence of a toxin-producing mold does not automatically imply the presence of mycotoxins in foods and feeds. Generally, three causes for contamination of foods are distinguished: a primary contamination of agricultural commodities in the field and upon postharvest storage, a secondary contamination during processing as a consequence of poor hygienic processing conditions, and finally, a carryover effect may occur with residues in animal-derived food via mycotoxin-contaminated feed.

Mycotoxin contamination of foods may cause considerable economic losses. On a global perspective, aflatoxins in tree nuts, dry fruits, and spices, *Fusarium* toxins in cereals (particularly maize, wheat, and barley), and ochratoxin A in cereals and coffee are of major importance (Bhat and Vasanthi, 1999). Regional problems also may arise from mycotoxins in fruits such as patulin in apples, ochratoxin A in grapes and dried vine fruits, or aflatoxins in different dried fruits.

The aim of this chapter is to summarize and critically discuss scientific data on mycotoxins in fruits. After giving an introduction on mold spoilage of fruits in general and factors affecting growth and mycotoxin formation by

molds, emphasis is placed on mycotoxin contamination of fruit products destined for human consumption. Strategies for preventing a possible post-harvest mycotoxin contamination and decontamination strategies for fruit products are then described. Based on data on the occurrence of mycotoxins in fruits and fruit products, the impact of mycotoxins in fruits on human health is also discussed.

II. MOLD SPOILAGE OF FRUITS

The food technological term *spoilage* covers "any chemical or physical alteration of food that makes it unfit or unsafe to eat" (Morris, 1992). *Plant-rot diseases* are characterized by "the softening, discoloration, and often disintegration of a succulent plant tissue as a result of fungal or bacterial infection" (Arneson and Hodge, 2004). Fruit rots are usually subdivided into field rots, which damage the plants before harvest, and storage rots, which occur after harvest. Rots are caused by multiple interacting factors, including those from bacterial or fungal pathogens or a complex of several pathogens under a diverse range of environmental factors (McManus, 2004). The most common rot pathogens are summarized in Table I.

Because most fruits contain the seeds of permanent crops like bushes and trees, which grow in orchards and yards for several years to decades, crop rotation, a feasible measure to control toxigenic fungi in agricultural and vegetable crops, is not applicable in fruit orchards and yards. Tillage and other soil cultivation systems that play an important role in the reduction of the infective potential of toxigenic fungi in agricultural soils are only applicable between the rows of fruit bushes and trees and, thus, are significantly less effective in orchards. The most important method to control the growth of mycotoxigenic fungi on fruits in the field and in storage devices is the application of fungicides (mostly synthetic) that more or less specifically inhibit mold growth and, thus, the production of mycotoxins. Fungicides are sprayed rarely to control the synthesis of mycotoxins and, thus, are an important determinant of yield quality directly, but they are applied in most cases to improve the yield or visible quality of fruits.

A. *ASPERGILLUS*

One of the most ubiquitous fungal genera is the genus *Aspergillus. Aspergillus* species grow like the other molds saprophytically on a wide range of organic substrates. The many species of the genus are arranged in sections, and the taxonomic situation of the sections, especially *Circumdati* and *Nigri,*

TABLE I
COMMON MOLD ROT DISEASES OF FRUITS AND THE MOLDS ASSOCIATED WITH THEM[a]

Commodity	Disease	Most important mold species
Citrus fruits	Blue rot	*Penicillium italicum (Penicillium ulaiense)*
	Green rot	*Penicillium digitatum*
	Sour rot	*Geotrichum candidum*
	Grey rot	*Alternaria alternata*
	Black rot	*A. alternata*
Apples	Brown rot	*A. alternata, Aspergillus niger, Aspergillus flavus*
Pome fruits	Blue rot	*Penicillium expansum*
	Grey rot	*Botrytis cinerea*
Stone fruits	Brown rot	*Monilia fructicola*
	Transit rot	*Rhizopus stolonifer, Rhizopus oryzae*
Melons	Black rot	*A. alternata*
	Pink rot	*Trichothecium roseum*
Figs	pink rot/soft rot (endosepsis)	*Fusarium moniliforme*
	Smut	*A. niger*
Grapes	Grey rot	*B. cinerea*
	Black rot/sour rot	*A. niger, Aspergillus carbonarius*
Berries		*B. cinerea, R. stolonifer, Mucor piriformis*

[a]Data from Logrieco *et al.* (2003) and Pitt and Hocking (1997).

is in a constant state of flux (Kusters van Someren *et al.*, 1991; Varga *et al.*, 2000; Yokoyama *et al.*, 2001). Many of the species mentioned in this chapter may have had different names or will have different names in the future.

Most fruits grown in the tropical and subtropical regions of the earth seem to be contaminated with *Aspergillus* species because they are ideally adapted to the climatic conditions prevailing in these regions. The growth of *Aspergillus* species of the section *Nigri,* for instance, starts above 10°C. The *A. niger* growth optimum is higher than 37°C, whereas that of *Aspergillus carbonarius* is consistently lower (Battilani *et al.*, 2003b). *A. carbonarius* optimum growth occurred at 35°C for all isolates tested by Mitchell *et al.* (2003). No growth occurred below 15°C. The optimum water activity (a_w) of the isolates varied between 0.93 and 0.987, with the widest a_w tolerance occurring at 25°C.

A wide spectrum of toxigenic molds, primarily belonging to the genera *Aspergillus* and *Penicillium,* were isolated from Egyptian strawberries, apricots, plums, peaches, grapes, dates, figs, apples, pears, and mulberries (Table II).

TABLE II

ASPERGILLUS SPECIES IDENTIFIED FROM FRESH FRUITS

Mold	Commodity	Region	Reference
A. flavus, A. parasiticus, A. tamarii, and *A. alliaceus*	Figs	California	Doster *et al.*, 1998
A. ochraceus, A. melleus, A. sclerotiorum, and *A. alliaceus*	Figs	California	Bayman *et al.*, 2002
A. niger var. niger, A. flavus, A. niger var. awamori, A. foetidus, and *A. candidus*	Grapes	Argentinia	Magnoli *et al.*, 2003
A. niger	Grapes	Brazil	Rosa *et al.*, 2002
A. carbonarius, A. section *Nigri, A. aculeatus, A. fumigatus, A. terreus*, and *A. ustus*	Grapes	France	Sage *et al.*, 2002
A. section *Nigri* and *A. carbonarius*	Grapes	Portugal	Serra *et al.*, 2003
A. niger, A. tubigensis, A. carbonarius, A. aculeatus, A. japonicus, A. ochraceus, and *A. fumigatus*	Grapes	Italy	Battilani *et al.*, 2003a
A. niger var. *niger* and *A. carbonarius*	Grapes	Spain	Belli *et al.*, 2002
A. niger, A. flavus, and *A. terreus*	Apples	Egypt	Hasan, 2000
A. flavus	Quinces	India	Sharma and Sumbali, 1999
A. niger, A. flavus	Dates	Egypt	Ragab *et al.*, 2001
A. candidus, A. flavus, A. niger, A. ochraceus, A. sclerotiorum, and *A. terreus*	Strawberries, apricots, plums, peaches, grapes, dates, figs, apples, pears, mulberries	Egypt	Aziz and Moussa, 2002

Besides nontoxigenic species, the toxigenic fungi *Aspergillus candidus, Aspergillus flavus, A. niger, Aspergillus ochraceus, Aspergillus sclerotiorum,* and *Aspergillus terreus* frequently were found (Aziz and Moussa, 2002). Mold isolates from brown-rot lesions of Egyptian apples included, besides fungi from other genera, the following *Aspergillus* species: *A. niger*, *A. flavus,* and *A. terreus* (Hasan, 2000). Belli *et al.* (2002) isolated the *A. niger* aggregates (formerly *A. niger* var. *niger*) and *A. carbonarius* from Spanish grapes. *A. flavus* also usually infects quinces (*Cydonia oblonga*) in the State of Jammu and Kashmir, and according to Sharma and Sumbali (1999), about 23% of the tested isolates were aflatoxigenic.

Several *Aspergillus* species infect figs. Doster and Michailidis (1998) detected aflatoxin-producing L and S strains of *A. flavus* and *A. parasiticus,* as well as the nonproducing species *A. tamarii* and *A. alliaceus* in Californian figs, whereas Bayman *et al.* (2002) detected *A. ochraceus, A. melleus,* and *A. sclerotiorum* from the section *Circumdati* and *A. alliaceus,* which seems to be more closely related to aspergilli from the section *Flavi*, on figs from California. *A. alliaceus*–infected figs contained ochratoxin A, whereas figs infected with isolates from the section *Circumdati* contained little or no toxin (Bayman *et al.*, 2002). The section *Circumdati* has taxonomic problems for *Aspergillus* species, because several species are poorly defined or may be synonymous, so a taxonomic revision of the section is needed (Varga *et al.*, 2000). Toxigenic strains of *A. flavus* are often found in molded fresh and dried fig products in the Mediterranean region (Logrieco *et al.*, 2003).

Grapes from tropical and subtropical regions also are regularly contaminated with *Aspergillus* species, whereas *Alternaria* was isolated most frequently from Argentinian grapes (80% of the samples), followed by *Aspergillus* (70%). In grapes, *Alternaria alternata* was the only species identified in the genus, followed by *A. niger* var. *niger* and *A. flavus, A. niger* var. *awamori, A. foetidus,* and *A. candidus* (Magnoli *et al.*, 2003). Grapes from Argentina and Brazil had *Aspergillus, Penicillium,* and *Botrytis* species isolated from them (Rosa *et al.*, 2002). Other genera identified (in decreasing order) were *Phytophthora, Moniliella, Alternaria,* and *Cladosporium.* Rosa *et al.* (2002) isolated 48 *A. niger* strains from Argentinian and 53 from Brazilian grape samples. They additionally identified *A. flavus* and *A. ustus* in Argentinian and *A. carbonarius, A. flavus, A. ochraceus,* and *A. terreus* in Brazilian grape samples. *A. flavus* and *A. terreus* did not produce ochratoxin A when cultured on nutrient medium, and of the remaining species, the percentage of toxigenic strains and the ochratoxin A levels produced were significantly lower than in *A. niger.* In 11 vineyards from four wine-making Portuguese regions, *Aspergillus* species from the section *Nigri* were predominant. Most of the *A. carbonarius* (97%) and 4% of the *A. niger* isolates tested produced ochratoxin A on nutrient agar plates (Serra *et al.*, 2003), confirming the hypothesis

that *A. carbonarius* is the main producer of ochratoxin A in grapes. Fifty samples of currants, raisins, and sultanas from the Spanish market were analyzed by Abarca *et al.* (2003) for mold contamination and 98% of the dried fruits had mainly black aspergilli and species from the order *Mucorales.* Among the *Aspergillus* section *Nigri*, *A. niger* var. *niger* was detected in 49 (98%) and *A. carbonarius* in 29 (58%) of the samples. Other species from the genus found in this study were *A. flavus* and *A. versicolor.* According to Sage *et al.* (2002), four of 11 French grape samples were contaminated by potentially ochratoxigenic strains of *A. carbonarius.* Other grape-infecting species from the genus were species from the *Aspergillus* section *Nigri, A. aculeatus, A. fumigatus, A. terreus,* and *A. ustus* in descending prevalence. Battilani *et al.* (2003b) collected 508 mold isolates from Italian grapes, with 477 isolates belonging to the genus *Aspergillus* and 31 to the genus *Penicillium.* Among the genus *Aspergillus,* species from the section *Nigri* largely predominated (464 isolates) and species from the sections *Circumdati* (*A. ochraceus*) and *Fumigati* (*A. fumigatus*) were found only once or twice, respectively. Among the species of the section *Nigri*, the biseriate species *A. niger* and *A. tubingensis* dominated, followed by *A. carbonarius* and the uniseriate species *A. aculeatus* and *A. japonicus*. Logrieco *et al.* (2003) concluded that toxigenic strains of *A. carbonarius* are often associated with the Mediterranean region and black rot of grapes. Greek grapes from Corinth raisin and wine-producing regions regularly were infected with *Aspergillus* species. Species from the *A. niger* section *Nigri* prevailed as causal agents of the sour rot of grapes, and particularly, *A. carbonarius* caused concern as the dominating ochratoxin A producer on grapes (Tjamos *et al.*, 2004).

Compared to studies on effects of fungicides on growth of mycotoxigenic molds in cereals, however, relatively little is known about fungicidal effects on molds that infect fruits. The effect of several fungicides on toxigenic *Aspergillus* species of the section *Nigri* that infect grapes in Greek Corinth raisin and wine-producing regions has been tested. According to Tjamos *et al.* (2004), fungicides with the active ingredients fludioxonil and cyprodinil showed the most efficient growth-reducing effect on *Aspergillus* section *Nigri* fungi, whereas cyprodinil and carbendazim were largely ineffective.

B. *PENICILLIUM*

The climatic requirements of *Penicillium* species are quite different from those of the genus *Aspergillus.* Whereas *Penicillium* species grow over a temperature range of 4–31°C, *Aspergillus* species require temperatures of 12–39°C (International Programme on Chemical Safety, 1990). *Penicillium* species frequently is found outside tropical and subtropical regions of the Earth.

Sage *et al.* (2002), for instance, detected the potential ochratoxin A producer *P. chrysogenum* in three of 11 French grape samples. Other grape-infecting *Penicillium* species detected by these authors were (in decreasing incidence) *P. brevicompactum, P. expansum, P. thomii, P. glabrum, P. purpurogenum, P. chrysogenum, P. miczinskii, P. atramentosum, P. griseofulvum, P. minioluteum,* and single isolates of *P. canescens, P. citreonigrum, P. citrinum, P. echinulatum, P. oxalicum, P. paxilli, P. rugulosum,* and *P. spinulosum. P. chrysogenum* was isolated from 22% of Argentinian grape samples collected in the Mendoza province by Magnoli *et al.* (2003), whereas *P. glabrum* and *P. crustosum* were detected occasionally. Apart from a wide spectrum of molds from other genera, *P. brevicompactum, P. chrysogenum, P. citrinum, P. aurantiogriseum, P. commune, P. expansum, P. oxalicum, P. griseofulvum, P. islandicum,* and *P. verrucosum* were isolated by Aziz and Moussa (2002) from Egyptian strawberries, apricots, plums, peaches, grapes, dates, figs, apples, pears, and mulberries. Pianzzola *et al.* (2004) detected 11 *P. expansum* and three *P. solitum* strains, which cause blue mold of apple, the most important postharvest disease in Uruguay. Brown rot of apple also is caused by *P. expansum* besides other fungal species (Hasan, 2000). According to Logrieco *et al.* (2003), molded fig products in the Mediterranean region are often contaminated by *P. expansum,* among other fungi.

C. *ALTERNARIA* AND OTHER MYCOTOXIGENIC GENERA

The worldwide occurrence of *Alternaria* and other more rarely detected toxigenic species is less well documented than the case for *Aspergillus* and *Penicillium* species. According to Magnoli *et al.* (2003), about 80% of grape samples from a winery in the Argentinian Mendoza province were contaminated with *A. alternata,* but the authors did not detect any other species of the genus. *A. alternata* may also cause brown rot of apple (Hasan, 2000). *A. alternata* was identified by this author as the predominate species in the pathogenic complex causing brown rot of apples in Egypt. *A. alternata* has been isolated by Aziz and Moussa (2002) from strawberries, apricots, plums, peaches, grapes, dates, figs, apples, pears, and mulberries purchased in Egyptian grocery stores. In addition to the predominant *A. alternata,* unidentified species of the genus *Fusarium* were isolated by Hasan (2000), among other molds as components of the pathogenic complex causing brown rot of apples in Egypt.

In conclusion, the most common mycotoxin-producing fruit-rot pathogens belong to the genera *Aspergillus, Penicillium, Alternaria,* and *Fusarium.* Their impact on human health is based primarily on their occurrence, and the toxicity profiles of their mycotoxins are thought to decrease in this order as well. Several species of the genus *Aspergillus* infect fruits in tropical and

subtropical orchards, whereas *Penicillium* species and *A. alternata* infect fruits in nearly every fruit-growing region on earth. Fruit rots are caused by a pathogenic complex, and the causal agents cannot be identified reliably with the naked eye.

III. POTENTIAL FOR MYCOTOXIN FORMATION AND OCCURRENCE OF MYCOTOXINS IN FRUITS

Toxin production is, more or less, a metabolic burden. Non–mycotoxin-producing mold strains may thus be favored in specific cases and may displace mycotoxin-producing strains through competition (Desjardins *et al.*, 1993). However, growth of mycotoxigenic molds does not necessarily mean the formation and presence of mycotoxins in fruits. It is well described that minimum a_w values for mold growth and mycotoxin formation differ for several species. Table III summarizes these differences for the most important mycotoxigenic fungi.

Furthermore, differences in optimum temperatures for growth and mycotoxin formation exist. For *Aspergillus* species, temperature optimum for mycotoxin formation is at 25–28°C, whereas optimum temperature for mold growth is between 30 and 35°C. In the field, factors such as temperature or humidity (a_w values) may influence production of a mycotoxin more than application of fungicides, which usually are not designed to directly inhibit or prevent the production of mycotoxins (Scientific

TABLE III
ENVIRONMENTAL CONDITIONS FOR GROWTH AND MYCOTOXIN PRODUCTION BY DIFFERENT TOXIGENIC MOLDS[a]

	Mold growth		Mycotoxin production
Fungus	Optimum temperature [°C]	Minimum a_w value	Minimum a_w value
Aspergillus flavus	35–37	0.80	0.84
A. parasiticus	30	0.84	0.87
A. ochraceus	28–35	0.77	0.85
A. carbonarius	35	—[b]	—[b]
Penicillium expansum	25–26	0.84	0.99
Alternaria alternata	25–28	0.85–0.88	—[b]

[a]Data from Corry, 1987; Mitchell *et al.*, 2003; Weidenbörner, 2000.
[b]Not determined.

Committee on Plants of the European Commission, 1999). In a summary, the committee concluded in 1999 that "there is no sufficient evidence that pesticides play a prominent and consistent role in preventing or inhibiting the production of mycotoxins by toxigenic fungi. However, it cannot be excluded that, in the future fungicides will be selected on the basis that they effectively can inhibit the production of mycotoxins."

A. AFLATOXINS

As reviewed by Logrieco (2003), aflatoxin B_1 and B_2 are mainly produced by *A. flavus* and *A. parasiticus,* with the latter also producing aflatoxins G_1 and G_2. Because of the aforementioned optimum conditions for mold growth and mycotoxin production, plant products from tropical or subtropical regions are particularly at risk for aflatoxin contamination resulting from infection with *Aspergillus* species in the field. Accordingly, scientific papers on aflatoxin contamination in fruits focus on fruits cultivated in warm climates like figs, dates, and citrus fruits. However, under adverse storage conditions, postharvest infection and mycotoxin contamination of other fruits also may occur.

The susceptibility of maturing figs to decay by aflatoxin-producing fungi has been investigated by Doster *et al.* (1998). Figs became more susceptible as they matured through the four developmental stages: green with eye (ostiole) closed, green with eye open, yellow, and brown. The mature brown figs were the most susceptible to decay by *A. flavus.* Aflatoxin analysis showed that brown figs had more than six times the aflatoxin of yellow figs and more than 30 times that of green figs. Wounding did not result in a significant increase in infections or aflatoxin concentration in mature brown figs compared to nonwounded figs. The authors suggested that damage to mature brown figs does not favor aflatoxin production, which might explain why insect damage to mature figs did not result in increased aflatoxin contamination in figs.

Twenty-five varieties of dates were examined at different maturation stages for total microbial counts, aflatoxins, and aflatoxigenic *Aspergillus* species and lactic acid bacteria by Shenasi *et al.* (2002). Microbial counts were high at the first stage of maturation (Kimri) and increased sharply at the second stage (Rutab), then decreased significantly at the final dried stage of maturation (Tamr). Aflatoxins were detected in 12% of the samples, although aflatoxigenic *Aspergillus* were detected in 40% of the varieties examined; all were at Kimri stage only. No aflatoxins or aflatoxigenic *Aspergillus* species were detected at the final edible stage of maturation. The absence of aflatoxins in fresh dates has also been described by Ragab *et al.* (2001). A possible explanation is the antifungal effect of date extract as

described by Shraideh *et al.* (1998). Exposure of yeast to 5% date extract showed evidence of weakening in the cell wall of the yeast, with indications of cell distortion and partial collapse in some cases. Increasing the concentration of date extract (20%, w/v) led to more drastic damage to the yeast, which resulted in cell lysis and concurrent leakage of cytoplasmic material with eventual cell death.

Natural occurrence of aflatoxins on oranges in Egypt was investigated by Ragab *et al.* (1999). Thirty-two percent of oranges collected from a local market contained either all four major aflatoxins or a combination of aflatoxin B_1 and aflatoxin G_1. By inoculation of surface-intact oranges with *A. parasiticus,* Ragab *et al.* (1999) showed that aflatoxin formation may occur in the peel of oranges in spite of their high content of oils. For *A. flavus,* it has been shown that citrus oil exhibits an antifungal action (Varma and Verma, 1987). Mycotoxin production of *A. parasiticus* on oranges is positively correlated with temperature and relative humidity (Ragab *et al.*, 1999). Aflatoxins diffused into the edible portion of the fruit but appeared to be degraded with time. A similar observation previously had been reported by Varma and Verma (1987) for orange juice. A decrease in aflatoxin production with time was observed for *A. flavus* grown on oranges and in orange juice, but the authors suggested that aflatoxins were degraded by the mycelium of *A. flavus* itself. The production of aflatoxins was maximum when the biomass reached its optimal value and rapidly declined after the mycelium started to autolyze.

Singh and Sumbali (2000) demonstrated that mature jujube fruits are a favorable substrate for infection and aflatoxin production by *A. flavus* strains. Among the mycoflora on the surface of jujube (*Ziziphus mauritiana*) *A. flavus* consistently was recorded during the entire period of fruit development. Fifty isolates of *A. flavus* isolated from the preharvest fruits caused extensive postharvest rot of mature jujube when inoculated. When all of these isolates were also screened for their aflatoxigenic potential in mature jujube, 54% of the isolates tested positive for different aflatoxins (B_1, B_2, G_1, and G_2) at levels ranging from 31 to 2874 μg/kg.

B. OCHRATOXIN A

Depending on the geographic region and climate, ochratoxin A is produced by *Penicillium* or *Aspergillus* species. In recent years, much work has focused on the identification of ochratoxin producing fungal species on grapes in different climatic regions. In the past, the ability to produce ochratoxin A was believed to be restricted to *A. ochraceus* and closely related species from the section *Circumdati* and to *P. verrucosum,* but it has become evident that species from the *Aspergillus* section *Nigri* also are able to produce

this mycotoxin. Abarca *et al.* (2001) speculate that other species may be additional sources for ochratoxin production in their natural environments.

The difference in temperature requirements for mold growth may explain the observation reported by Pietri *et al.* (2001), in which *Penicillium* species occur on grapes only at the beginning of ripening in contrast to *Aspergillus* species, which were constantly associated with grapes during ripening and harvest in Italy. *P. verrucosum* and *A. alutaceus,* formerly known as *A. ochraceus,* were supposed to be the most important producers of ochratoxin A in grapes. Battilani *et al.* (2003a) reported that aspergilli were dominant to penicillia, with the section *Nigri* predominating. *A. carbonarius* probably plays an important role because of the high percentage of positive strains and the amount of ochratoxin A produced on grapes. Aspergilli section *Nigri* were already present on grape bunches early in the season, and their frequency increased during later growth stages. A total of 48 *A. niger* strains were isolated from Argentinian grapes, of which eight could produce ochratoxin A, and 16 of 53 *A. niger* strains from Brazilian grapes produced ochratoxin A (Rosa *et al.*, 2002). A key role for *A. carbonarius* concerning ochratoxin A production in grapes and musts from France was also reported by Sage *et al.* (2002).

Similar results were reported from Magnoli *et al.* (2003), who analyzed the mycoflora of Argentinian grapes. Of 63 strains belonging to the *Aspergillus* section *Nigri* and tested for ochratoxin A production, 41.3% were producers; levels of ochratoxin A produced ranged from 2 to 24.5 ng/ml of culture medium. The presence of ochratoxigenic strains of the *Aspergillus* section *Nigri* may be an important source of ochratoxin A in grapes from tropical and subtropical zones. *P. purpurogenum* has also been isolated from grapes, but was not shown to produce ochratoxin A (Cabanes *et al.*, 2002).

C. PATULIN AND CITRININ

Patulin and citrinin are both produced by *Penicillium* species. Citrinin was first isolated from *P. citrinum,* from which it derived its name. Today, it is known that apart from *Penicillium* species like *P. citreonigrum, P. citrinum, P. expansum,* and *P. verrucosum*, citrinin is also produced by other species like *A. candidus, A. terreus,* and *Monascus purpureus* or *M. ruber* (Weidenbörner, 2001). The occurrence of citrinin is mainly associated with rice and other cereals, but citrinin has also been detected in raisins (Meister, 2003) and in apples (Martins *et al.*, 2002).

A large number of molds produce patulin. Patulin is primarily known as a toxin produced by *P. expansum* in foods but may also be formed by *P. claviforme, P. cyclopium, P. equinum, P. glandicola, P. commune, P. lapidosum, P. melinii, P. novaezeelandiae,* and *P. griseofulvum,* as well as

Aspergillus clavatus, A. giganteus, A. terreus, and *Byssochlamys nivea* (Davis and Diener, 1987).

Early studies report patulin occurrence on bananas, pineapple, grapes, peaches, apricots, plums, and tomatoes, but the authors do not present data on incidence and concentration of contamination (Frank *et al.*, 1976; Thurm *et al.*, 1979). Inoculation experiments showed that patulin may be produced by *Penicillium* species on a variety of foods and especially a variety of fruits, but the natural occurrence of patulin is limited to fruits and is mainly associated with apples and apple products. According to Weidenbörner (2001), two factors are responsible. Besides an inactivation of patulin by certain compounds in foods, patulin-producing molds represent only a low percentage of the total mold strains isolated from most foods ranging from 0.9% for cornmeal to 1.42% for European-style dry sausage. Furthermore, an antifungal activity of bacteria against *P. expansum* has been described. Florianowicz (2001) investigated the antifungal activity of 11 selected bacterial cultures and five microfungi in different phases of their growth in respect of their activity against *P. expansum*. Two *Bacillus* species, *B. megaterium* and *B. subtilis,* and three strains of the genus *Lactobacillus, L. casei, L. delbrueckii* subspecies *bulgaricus*, and *L. lactis* subspecies *cremoris,* were active against *P. expansum.* Inhibition of patulin production by *P. expansum* using fruit oils was observed by Hasan (2000). Complete inhibition of patulin formation by *P. expansum* grown on glucose-Czapek's-apple medium after seven days at 25°C was achieved in the presence of 0.2% lemon oil. A lower but still very strong inhibition was observed with 0.05% lemon oil or orange oil at both concentrations.

In rotten apples, *P. expansum* is the predominating mold. Strains of *P. expansum* cause blue mold rot of apples after infection following preharvest insect or storm damage of the surface tissue or damage caused by rough gathering, prolonged storage, or improper postharvest handling (Logrieco *et al.*, 2003). Available literature on the occurrence of patulin has been reviewed by Drusch and Ragab (2003). Up to 130 mg of patulin/kg have been detected in the lesions of apples, with no correlation between the size of the lesion and the patulin concentration (Beretta *et al.*, 2000).

Martins *et al.* (2002) investigated the natural co-occurrence of patulin and citrinin on 351 samples of seven apple varieties. The percentage of samples contaminated with patulin only was 68.6%, whereas contamination with citrinin only was 3.9%. Co-occurrence of both mycotoxins occurred in 19.6% of the samples. The maximum mean patulin concentration was 80.5 mg/kg for Richared variety, and the maximum mean citrinin concentration was 0.92 mg/kg for Rome beauty variety. Because the ratio of weight of the rotten area to the total weight was about one-third, a direct risk for mycotoxin ingestion for consumers seems unlikely. Apples with such a high

proportion of rotten tissue are usually not consumed or processed. Demirici *et al.* (2003) detected patulin in cherries, mulberry, raspberry, and strawberry. In 31 of 41 samples of fruits collected from the Turkish market, these authors found up to 746 μg of patulin/kg in raspberries, up to 572 μg of patulin/kg in strawberries, and up to 426 μg/kg in mulberries. Cherries were contaminated most frequently with patulin, 9 of 10 samples contained patulin, with a mean concentration of 37 μg/kg.

D. *ALTERNARIA* TOXINS

Scott (2001) summarized the available literature on the natural occurrence of *Alternaria* toxins in fruits. The most frequently detected toxins were alternariol, alternariol methyl ether, and tenuazonic acid in apples, mandarin, melon, and alternariol and its methyl ether in red currant, raspberry, strawberry, gooseberry, and blackberry. Stinson *et al.* (1980) were able to detect *Alternaria* toxins after the authors isolated *Alternaria* strains from blueberries, broke the skin, and inoculated the berries after steam disinfection. In contrast, Tournas and Stack (2001) did not detect *Alternaria* toxins after infection of blueberries with *A. alternata.* As discussed earlier in this chapter, fruits become more susceptible to mold invasion during ripening, and this may be a crucial point for inoculation experiments.

Depending on the substrate and the strain of *A. alternata,* the mycotoxin profile produced may vary. Black and gray strains of *A. alternata* produced tenuazonic acid, alternariol, and alternariol methyl ether, when grown on rice substrate or whole mandarin fruits. Gray strains of *A. alternata* also produced altertoxin I, and when cultivated on autoclaved rice, both strains were able to produce altenuene (Logrieco *et al.*, 1990). Investigations carried out on the natural occurrence of mycotoxins in infected fruits showed that samples of the two kinds of mandarin heart rot showed different mycotoxin profiles. In black-rot samples, tenuazonic acid, alternariol methyl ether, and alternariol (up to 87, 1.4, and 5.2 mg/kg) were found, whereas tenuazonic acid (up to 174 mg/kg) was the only detectable mycotoxin in gray-rot samples (Logrieco *et al.*, 1990). Traces of tenuazonic acid have been reported by Bottalico and Logrieco (2001) for samples of black-rot melons.

Tournas and Stack (2001) investigated mycotoxin formation by two *Alternaria* strains (ATCC 66868 and ATCC 56836) cultivated on strawberries, grapes, and apples at various temperatures ranging from 5 to 21°C. *A. alternata* and its toxins were not a major problem in strawberries because of the presence of fast-growing molds like *Botrytis* and *Rhizopus* that overgrew and inhibited *Alternaria*. Growth of *Alternaria* species and mycotoxin formation may also limit storage stability of fruits. Alternariol and alternariol methyl ether were detected in grapes even when stored at 5°C, whereas

in apples, a temperature of more than 10°C was required for mycotoxin formation. Alternariol methyl ether concentration in apples was up to 14 mg/kg, which may constitute a problem when apples are stored for extended periods of time during the winter and spring season under uncontrolled atmosphere (Tournas and Stack, 2001). Scott (2001) pointed out that more work is needed on the development of reliable methods for the determination of *Alternaria* toxins. However, data from the studies cited give sufficient evidence that *Alternaria* toxins frequently occur and that the occurrence may not be limited to fruits. Generally, the presence of *Alternaria* toxins in fruit products serves as an indicator of the use of poor-quality raw materials.

E. RARELY DETECTED TOXINS

A detailed mycotoxin profile of a *Penicillium vulpinum* strain isolated from apples (formerly *P. claviforme*) was described by Kozlovskii *et al.* (2000). The authors detected viridicatin, cyclopenin, and a-cyclopiazonic acid, but the strain did not produce detectable levels of patulin and citrinin. Venkatasubbaiah *et al.* (1995) detected by thin-layer chromatography, high-performance liquid chromatography (HPLC), mass spectrometry (MS), and ^{1}H nuclear magnetic resonance spectrometry, among other toxic substances, the trichothecene mycotoxins trichothecolone and trichothecolone acetate in liquid cultures of *Peltaster fructicola,* one of the molds that cause sooty blotch of apple. Feldmann *et al.* (2003), however, were unable to confirm the results for different strains of *P. fructicola* with a more sensitive HPLC/MS/MS system. The authors discussed the trichothecene identification of Venkatasubbaiah *et al.* (1995) as possibly resulting from HPLC matrix disturbances or delay phenomena.

Jiménez and Mateo (1997) screened corn and rice cultures of five species of *Fusarium* originally isolated from banana. In these cultures, they detected the type A trichothecenes deoxynivalenol (DON) and 3-acetyl DON in *F. graminearum,* the type B trichothecenes T-2 tetraol and neosolaniol in *F. acuminatum,* zearalenone in *F. graminearum* and *Fusarium equiseti,* a-zearalenol in *F. equiseti,* and fumonisin B1 in *F. moniliforme* and *F. proliferatum.* Moretti *et al.* (2000) isolated 120 *F.* strains from rotted fig fruits mainly belonging to the species, *F. ramigenum, F. solani,* and *F. subglutinans*, and at a lower frequency, *F. proliferatum*. Cultivated on maize kernel media, fusaric acid was produced by all species at very low amounts, but one strain of *F. subglutinans* produced a high level of this toxin. *Liseola* section species produced beauvericin, fumonisins, and fusaproliferin. As reviewed by Logrieco *et al.* (2003), exposure of plants to *Fusarium* toxins and their relative toxicological risk are not well understood. For example, toxigenic *Fusarium* species are important pathogens and root colonizers of various fruits

(e.g., banana, mango, and pineapple) and vegetables (e.g., potato), but no reports of the natural occurrence of *Fusarium* mycotoxins in products obtained from these plants exist.

In conclusion, the growth of mycotoxigenic mold species on fruits is not always an indication of the presence of mycotoxins, but they may also be absent due to the growth of nontoxigenic fungal strains. Temperature and humidity may significantly affect mycotoxin formation in the field or after harvest, and fungicides may be applied to prevent or inhibit mold growth. Because aflatoxins occur in the field only in tropical and subtropical regions, figs, dates, and citrus fruits should be monitored with special attention for signs of mold growth. Ochratoxin A, patulin, citrinin, and the toxin mixture of *A. alternata* may be present in fruits from nearly every fruit-growing region of the Earth, but it should be considered that several of the toxin-producing species have a relatively narrow host range.

IV. MYCOTOXINS IN FRUIT PRODUCTS AND IMPACT OF PROCESSING ON MYCOTOXIN CONCENTRATION

The effects of postharvest storage and processing of fruits on fungal growth and a possible contamination with mycotoxins after harvest is of special interest. Fruits should be harvested at optimum maturity and should be handled gently to prevent bruises and punctures that permit fungal invasion. Good sanitation should be maintained, moldy fruits and fruits with skin breaks and bruises should be culled out, and washing with hot water or fungicide treatments may also be applied (Splitstoesser, 1987). However, the application of fungicides to fruits after harvest to reduce decay has been increasingly curtailed by the development of pathogen resistance to many key fungicides, the lack of replacement fungicides, negative public perception regarding the safety of pesticides, and consequent restrictions on fungicide use. Biological control of postharvest diseases has emerged as an effective alternative. Because wound-invading necrotrophic pathogens are vulnerable to biological control, antagonists can be applied directly to the targeted area (fruit wounds), and a single application can significantly reduce fruit decays as reviewed by Janisiewicz and Korsten (2002).

In terms of product safety, diffusion of mycotoxins in infected fruits and possible health risks associated with the processing or consumption of the remainder of an infected fruit after removal of visibly rotted tissue is of interest. Laidou *et al.* (2001) investigated the diffusion of patulin in the flesh of pears inoculated with four pathogens, *P. expansum, A. flavus, Stemphylium vesicarium,* and *A. alternata*. *P. expansum* and *A. flavus* penetrated more rapidly into the flesh than *S. vesicarium* and *A. alternata* because of the

utilization of substrate or the mechanism of breaking host defense. The selected strain of *A. flavus* did not produce patulin. Patulin diffused up to 6 mm in sound tissues of pears inoculated with *P. expansum* or *A. alternata* and up to 18 mm in sound tissues of pears inoculated with *S. vesicarium,* indicating that the absence of rots does not guarantee an absence of the mycotoxin (Laidou *et al.*, 2001), but diffusion is limited to the vicinity of rotted parts of the fruit. Patulin did not diffuse more than 2 cm from the infected zone into apples inoculated with *P. expansum* as determined by a stable isotope dilution assay (Rychlik and Schieberle, 2001). In contrast, in tomatoes, diffusion of patulin occurred throughout the whole fruit. The authors predicted that patulin may easily penetrate through low-viscous foods containing high amounts of water like blueberries, grapes, or melons. Similar results have been reported for ochratoxin A by Engelhardt *et al.* (1999) in different visibly rotted fruits after removal of rotted areas.

This approach gives only qualitative information about diffusion of mycotoxins into sound tissue and only in cases in which diffusion occurs. Freedom from ochratoxin A in sound tissue may result from the absence of ochratoxin A in the rotted area or from lack of diffusion. On the other hand, a positive result for ochratoxin A in sound tissue gives proof of diffusion. Ochratoxin A was detected in the sound tissue of cherries, strawberries, and tomatoes, but not in nectarines and apricots (Engelhardt *et al.*, 1999). In peaches with cleaved moldy stones, Engelhardt *et al.* (1999) detected up to 0.21 μg of ochratoxin A/kg, and its frequency of contamination was up to 50%. In conclusion, trimming of fruits can reduce mycotoxin content to a certain degree, depending on the type of fruit. Consumers should keep in mind that visibly sound fruits after trimming may still contain mycotoxins. Nevertheless, for industrial processing of certain fruits like apples, trimming is a useful tool for reduction of possible mycotoxin contamination and, therefore, leads to an increase in product safety.

As stated by Scott (1984), published information on mycotoxins in foods processed for human consumption is limited. Information on the extent that mycotoxins persist through processing is important for risk management by food processors and regulatory authorities. Whereas past research mainly focused on cereal processing, this chapter summarizes available literature on major categories of fruit products: dried fruits, fruit juices, wine and cider as fermented fruit juices, and fruit purees and jams.

A. DRIED FRUITS

The most important dried fruits produced for human consumption are raisins, sultanas, figs, apricots, and dates. Because all these fruits are cultivated in warm climates, mycotoxins associated with these fruits are aflatoxins and

ochratoxin A. Aflatoxins in figs are mainly produced by *A. flavus* or *A. parasiticus* (Doster and Michailides, 1998). To determine the likely origin of ochratoxin A in dried vine fruits, a mycological study of 50 samples (currants, raisins, and sultanas) from the Spanish market was conducted by Abarca *et al.* (2003), who found that 96.7% of *A. carbonarius* isolates and 0.6% *A. niger* varietas *niger* isolates were ochratoxin producers. Among the black aspergilli, *A. carbonarius* showed a consistent ability to produce ochratoxin A and is, thus, considered the most probable source of ochratoxin A contamination in dried vine fruits.

Few data on mycotoxin contamination of dried fruits (raisins, figs, prunes, dates, and apricots) have been published. Table IV shows the most recent surveys on the occurrence of ochratoxin A and aflatoxins in dried fruits. The frequency of contamination varies widely from 0 to 57% for aflatoxins and from 20% to 95% for ochratoxin A, respectively. In dried vine fruits, high concentrations of ochratoxin A up to 53.6 μg/kg have been reported. Mean ochratoxin levels in dried vine fruits were generally lower and ranged from 1 to 3 μg/kg. Stefanaki *et al.* (2003) reported that sultanas were less contaminated than currants. Aflatoxin contamination of dried fruits is usually below 2.5 μg/kg. High aflatoxin levels in dried figs of up to 1342 μg/kg have been found by Waizenegger (2001); however, the fruits were sorted before analysis, and data, therefore, reflect contamination of individual figs rather than the contamination level of a whole batch. The data of Waizenegger (2001) exemplify that individual moldy fruits may be highly contaminated with mycotoxins and that contamination in whole batches of dried fruits may be at considerably lower levels.

Mycotoxin contamination of dried fruits may start with fungal contamination on the trees, increase during harvesting and sun drying, and continue to accumulate during storage because of rewetting and improper storage conditions. Factors influencing mold growth and subsequent mycotoxin formation, and possible preventive measures have been reviewed by Drusch and Ragab (2003). These authors mention that several factors, such as cultivar susceptibility to fungal invasion, environmental stress conditions like drought, insect activity, mechanical damage, nutritional deficiencies, temperature, and humidity during growth determine possible mycotoxin contamination of the final product. Plant pathogens may infect a variety of tissues in addition to the fruits, and, thus, the removal of diseased branches and other plant parts helps to reduce the incidence of spoilage. Cultural practices that lower humidity in the growing area, such as weed control and the proper spacing of plants, often have a beneficial effect because growth of fungi is favored by moist conditions. Sanitation in the orchard and vineyard is important for control of spoilage organisms that overwinter in cankers, dead branches, and fallen fruit (Splitstoesser, 1987).

TABLE IV

DATA FROM RECENT SURVEYS ON AFLATOXINS AND OCHRATOXIN A IN DRIED FRUITS

Mycotoxin	Commodity	Total no. of samples (n)	No. of positive samples n (%)	Maximum (μg/kg)	Median (μg/kg)	Mean (μg/kg)	Reference
Ochratoxin A	Raisins, sultanas, currants	60	53 (88)	53.6	—	—	MacDonald *et al.*, 1999
	Raisins, currants	106	101 (95)	21.4	0.32	—	Engel, 2000
	Raisins, currants	59	10 (17)	19.0	0.92	2.64	Möller and Nyberg, 2003
	Raisins, currants	59	12 (20)	34.6	0.2	1.17	Möller and Nyberg, 2003
	Currants, sultanas	81	60 (74)	13.8	1.2	2.6	Stefanaki *et al.*, 2003
	Dried vine fruits	300	220 (73)	21.3	0.7	2.0	Food Standards Agency, 2003a
Ochratoxin A	Figs	34	27 (79)	3.95	0.02	—	Engel, 2000
Ochratoxin A	Prunes	31	26 (84)	0.07	0.03	—	Engel, 2000
Aflatoxins B_1, B_2, G_1, G_2	Raisins, sultanas, currants	60	0 (0)	—	—	—	MacDonald *et al.*, 1999
Aflatoxin B_1	Raisins	100	2 (2)	300	260	260	Youssef *et al.*, 2000
Aflatoxins B_1, B_2, G_1, G_2	Figs	42	7 (17)	1342	215	—	Waizenegger, 2001
	Figs	21	12 (57)	<2.5	—	—	Food Standards Agency, 2002
Aflatoxins B_1, B_2, G_1, G_2	Prunes	16	5 (31)	<2.2	—	—	Food Standards Agency, 2002
Aflatoxins B_1, B_2, G_1, G_2	Dates	12	6 (50)	<2.5	—	—	Food Standards Agency, 2002
Aflatoxins B_1, B_2, G_1, G_2	Apricots	12	1 (8)	<2.2	—	—	Food Standards Agency, 2002

Other key elements of preharvest management are cultivar selection, judicious use of insecticides and fungicides, and if necessary irrigation. Different fungicides (copper oxychloride, mancozeb, benomyl, captan, thiram, chlorothalonil, and prochloraz) were successfully applied for reducing *A. flavus* and *A. parasiticus* in dried figs and subsequently aflatoxins (Tosun and Delen, 1998). Maturation stages were taken into consideration for application time and type of fungicides. Treatment types involved tree and soil applications at wintering and budding stages, respectively, to reduce possible fungal sources on the trees and treatment at fruiting, at ripening and at shriveling. The latter were applied only to the soil under the trees where dried figs dropped to eradicate the soil-borne fungi that are mostly present in the topsoil level. A last form of treatment was applied to both the soil surface of the drying place and to the storage room before the fruits were stored.

Splitstoesser (1987) summarized some important control practices at harvesting, grading, and packing stages as follows: (1) Harvest fruit when at optimum maturity, (2) handle the fruit gently to prevent bruises and punctures that would permit the entry of saprophytic fungi, (3) maintain good sanitation to minimize the buildup of mold on fruit-contact surfaces. Mechanical harvesters, lug boxes, and packinghouse equipment should be cleaned and sanitized regularly. Live steam, formaldehyde, and fumigation with chlorine gas were some of the treatments used to destroy fungal spores. (4) Moldy fruit and those showing skin breaks and bruises should be culled out during the sorting and grading operations.

Drying of the fruits is usually carried out directly in the orchard. Raisins are harvested from the grapevine and sun-dried on the ground. It is important to prevent direct contact of the grapes with the soil to hamper fungal colonization on the fruits. In contrast, figs are left on the trees until they shrivel. The figs fall to the ground and are gathered up and dried further in the sunlight on drying devices for about five days. After drying, the sound fruits are stored in farmer storehouses.

The most detailed investigation on the influence of harvesting and drying techniques on mycotoxins in dried figs has been published in 1995 by Özay *et al.* (1995). Apart from classic sun-drying as described earlier, the authors investigated the effectiveness of solar drying. For solar drying, radiation energy from the sun was used for heating air up to a temperature of 60–65°C. The air was forced through a drying chamber, in which figs were placed on a wire mesh. In solar-dried figs, moisture content and a_w value (15.3% and 0.566%, respectively) were lower than in sun-dried figs. Özay *et al.* (2005) concluded that picking fruits from the tree and application of solar drying were the most effective treatments to reduce fungal contamination in dried figs. Waizenegger (2001) investigated a possible correlation between fluorescence and aflatoxin contamination and its use for reducing

aflatoxins in dried figs. The fluorescence, bright green-yellowish under ultraviolet light, is caused by kojic acid, a metabolite of certain *Aspergillus* species. Doster and Michailides (1998) reported that bright green-yellowish fluorescence in naturally infected figs was associated with decay by only four fungal species: the aflatoxin-producing species *A. flavus* (both L and S strains) and *A. parasiticus,* and the aflatoxin non-producers *A. tamarii* and *A. alliaceus.* Figs infected with *A. flavus* or *A. parasiticus* and showing no bright green-yellowish fluorescence were occasionally contaminated with aflatoxins, whereas other figs showing bright green-yellowish fluorescence and infected with *A. flavus* or *A. tamarii* had no aflatoxins. In a different study, Steiner *et al.* (1988) found that removal of bright green-yellow fluorescent figs from a 56-kg batch effectively lowered the original contamination level from 22.6 to 0.3 μg/kg aflatoxin B_1. All 52 bright green-yellow–positive figs contained kojic acid in concentrations between 8 and 6900 mg/kg. Thirty-seven (71%) fruits were contaminated with aflatoxin B_1 in concentrations between 5 μg and 76 mg/kg, and 15 (29%) fruits were contaminated with aflatoxin G_1 at levels of 5 μg to 180 mg/kg.

However, fluorescence was sometimes only detectable after cutting of the fruits (Doster and Michailides, 1998; Waizenegger, 2001). Nevertheless, screening of figs for bright green-yellowish fluorescence is a possibility for reducing aflatoxin contamination in dried fruits for human consumption, although acceptable quality-control measures for routine analysis should be nondestructive. Doster and Michailidis (1998) pointed out that bright green-yellow fluorescence might be of use to remove aflatoxin-contaminated figs for certain specific situations in California such as the reduction of aflatoxin contamination during the manufacturing of fig paste, because the figs are cut into quarters during processing.

B. FRUIT JUICE

Apples and pears are the fruits most frequently contaminated with patulin. As a consequence, most scientific publications on mycotoxin contamination of fruit juices deal with the occurrence of patulin in apple juice. Table V shows the most important surveys on the occurrence of patulin in apple juice for the last five years. The frequency of contamination ranged from 13 to 81%. Apart from one Turkish study with a mean of 140 μg/L (Yurdun *et al.*, 2001), mean patulin concentrations were rather low and usually below 50 μg/L. The highest patulin concentration was 733 μg/L, but concentrations up to 1150 μg/kg have occasionally been reported for commercially available apple juice (Beretta *et al.*, 2000).

Beretta *et al.* (2000) also observed a statistically significant difference between apple juices produced from conventionally and organically grown

TABLE V
DATA FROM RECENT SURVEYS ON PATULIN CONTAMINATION IN APPLE JUICE

Origin of samples	Unit	Total no. of samples (n)	No. of positive samples n (%)	Maximum	Mean	Median	Reference
United Kingdom	µg/L	199	110 (55)	171	14.3	7.1	European Commission (Directorate-General Health and Consumer Protection), 2002b; Food Standards Agency, 1999
Taiwan	µg/L	71	12 (17)	39.9	–	–	Lai *et al.*, 2000
South Africa	µg/L	31	8 (26)	45	–	–	Leggott and Shephard, 2001
Turkey	µg/L	45	27 (60)	732.9	139.9	–	Yurdun *et al.*, 2001
Sweden	µg/kg	39	5 (13)	<50	–	–	Thuvander *et al.*, 2001
Austria	µg/kg	242	114 (47)	50	14.9	1.6	European Commission (Directorate-General Health and Consumer Protection), 2002b
Belgium	µg/kg	117	27 (23)	59	16	<LOQ	
France	µg/kg	67	37 (55)	130	14.3	3.0	
France	µg/kg	122	49 (40)	37	11.6	1.7	
Germany	µg/kg	1248	320 (26)	415	22.1	4.2	
Turkey	µg/L	30	12 (40)	106.9	35.1	16.8	Demirci *et al.*, 2003
Italy	µg/L	44	11 (25)	74.2	26.7	–	Riteni, 2003
Belgium and imported	µg/L	43	35 (81)	38.8	9.0	6.0	Tangni *et al.*, 2003

apples. The highest value for apple juice from conventional agriculture was 3.0 μg/kg and for apple juice from organic agriculture was 28.2 μg/kg. These authors suggest that the application of fungicides in conventional agriculture was the reason for the lower contamination. Apart from the use of fungicides, patulin content in apple juice is significantly determined by the quality of apples as influenced by harvesting and storage conditions, by the different processing steps during juice production and by other juice ingredients.

The quality of apples for juice production is determined by the proportion of rotted and decayed apples, because patulin concentration in the brown-rotten area of apples is usually very high. Sydenham *et al.* (1997) reported up to 6.3 mg of patulin/kg in the rotted areas of apples; however, Beretta *et al.* (2000) detected up to 113 mg of patulin/kg in the same areas.

Kadakal and Nas (2002b) used apples, classified by the decay proportion on the fruit surface as sound, 30, 60, or 100% decayed, in the production of apple juice, to determine the effect of apple decay proportion on the patulin content of apple juice. Patulin increased in apple juice samples as the decay proportion increased. Patulin in juice samples produced with apples that were sound, 30, 60, and 100% decayed, were 0–15.9, 47.1–500.3, 156.4–2257.5, and 54.9–2508.6 μg/kg. Similar results have been reported by Jackson *et al.* (2003). Patulin was not detected in juice pressed from fresh tree-picked apples but was found at levels of 40.2–374 μg/L in juice pressed from fresh ground-harvested (dropped) apples. Another possible source of patulin contamination may be contamination of apple juice with *P. expansum*. McCallum *et al.* (2002) observed extensive fungal growth and high patulin levels after inoculation of apple cider with different isolates of *P. expansum.* Concentrations of 538–1822 μg/ml in apple ciders were associated with incubation at room temperature (25°C), and potentially toxic patulin levels of 75–396 μg/ml also were found in refrigerated ciders (4°C) inoculated with *P. expansum*.

Removal of rotten fruits by sorting is an effective technique to reduce possible patulin contamination in apple juice. Jackson *et al.* (2003) did not detect patulin in juice pressed from tree-picked apples stored for 4–6 weeks at 0–2°C after sorting but found it at levels of 0.97–64.0 μg/L in juice pressed from unculled fruits stored under the same conditions. Cider from controlled-atmosphere–stored apples that were culled before pressing contained 0–15.1 μg/L of patulin, whereas cider made from unculled fruit contained 59.9–120.5 μg/L of patulin. The importance of removing contaminated apples from the initial processing line during apple juice production was studied during three consecutive seasons by Leggott *et al.* (2000). Patulin concentration of 440 μg/kg was significantly reduced to 200 μg/kg after removal of rotten and damaged apples. Sydenham *et al.* (1997) investigated the effect of storage in the open on patulin content in apples and

apple juice. Storage of apples in the open is unavoidable in countries in which only limited storage facilities for cold or modified atmosphere storage are available. Over a period of 33 days, mean patulin levels in unprocessed fruits increased from 90 to 2445 μg/kg but decreased to between 75 and 695 μg/kg following a water wash step. Subsequent removal of rotten fruit decreased patulin levels further to between 55 and 405 μg/kg.

After washing, sorting, and pressing, raw apple juice usually is clarified to remove suspended solids. For this purpose, juice is treated with pectinolytic enzymes and/or nonenzymatic fining agents like bentonite, gelatine, caseinate, or chitosan. Solid particles are removed by centrifugation, filtration or ultrafiltration. Bissessur *et al.* (2001) evaluated the effectiveness of several clarification processes, namely clarification with bentonite, enzymatic (pectinase) treatment, paper filtration, and centrifugation for the reduction of patulin. Pressing followed by centrifugation resulted in an average toxin reduction of 89%. Total toxin reduction using filtration, enzymatic treatment, and fining were 70, 73, and 77%, respectively. Patulin reduction was due to the binding of the toxin to solid substrates that were verified by analyzing the clarified juice as well as the filter cake, pellet, and sediment.

Table VI gives an overview on the effect of single processing steps and treatments on patulin content in apple juice. In agreement with the observation of Bissessur *et al.* (2001), washing and pressing are the most effective steps for reducing patulin concentration in apple juice. Washing of ground-harvested apples before pressing reduced patulin levels in juice by 10–100%, depending on the initial patulin levels and the type of wash solution used (Jackson *et al.*, 2003). Hence, patulin is a good indicator of the quality of the apples used to manufacture juice. It is recommended that avoidance of ground-harvested apples and the careful washing and sorting of apples before pressing are good methods for reducing patulin levels in juice. As a consequence, the Codex Committee on Food Additives and Contaminants (CCFAC) presented a draft for a "Code of practice for the prevention of patulin contamination in apple juice and apple juice ingredients in other beverages" (Codex Alimentarius Commission, 2002). According to the CCFAC, postharvest management systems based on HACCP for the reduction of patulin in apple juice should be considered.

Table VI also shows that the use of activated charcoal seems to be another effective step in reducing patulin contamination in apple juice. Leggott *et al.* (2000) observed a reduction of patulin from 110 to 75 μg/L by a combination of depectinization, charcoal treatment, and ultrafiltration, which was probably because of the adsorption of patulin on the activated charcoal. No further removal of patulin occurred during the remainder of the juicing process. Leggott *et al.* (2001) reported that the type of charcoal

TABLE VI
REDUCTION OF PATULIN CONTAMINATION BY INDIVIDUAL PROCESSING STEPS DURING APPLE JUICE PRODUCTION

Treatment	Initial concentration	Reduction (%)	Reference
Washing	90/345/2445 μg/kg	17/75/72	Sydenham *et al.*, 1997
	47–339 μg/kg	21–31	Acar *et al.*, 1998
	2010 μg/kg	78	Leggott *et al.*, 2000
	<60–374 μg/L	10–100[a]	Jackson *et al.*, 2003
Pressing	2000 μg/L	52.5	Bissessur *et al.*, 2001
Centrifugation	2000 μg/L	36.5	Bissessur *et al.*, 2001
Clarification	47–339 μg/kg	15–49	Acar *et al.*, 1998
(gelatine/bentonite)	2000 μg/L	24.5	Bissessur *et al.*, 2001
Enzymatic treatment (depectinization)	2000 μg/L	20.5	Bissessur *et al.*, 2001
Filtration	2000 μg/L	17.5	Bissessur *et al.*, 2001
Depectinization, limited clarification and ultrafiltration	163–313 μg/kg	19–29	Acar *et al.*, 1998
Activated charcoal	120 μg/L	45–80	Leggott *et al.*, 2001
	117 μg/kg	23	Kadakal *et al.*, 2002
	62.3 μg/kg	57	Kadakal and Nas, 2002a

[a]Calculated based on the differences in patulin level in the final product.

used for patulin reduction had to be carefully selected. Different steam-activated carbons exhibited similar adsorption isotherms at a dosage level of 1 g/L and achieved patulin reduction rates of 70–80%. In contrast, chemically activated carbon was less effective in removing patulin and achieved only a 45% reduction at a dose of 1 g/L. Huebner *et al.* (2000) developed an ultrafine activated carbon bonded onto granular quartz to produce a composite carbon adsorbent with a high carbonaceous surface area, good bed porosity, and increased bulk density for the reduction of patulin levels from aqueous solutions and apple juice. Fixed-bed adsorption with 1 g of composite carbon adsorbent was also effective in reducing patulin concentrations (20 μg/L) in a naturally contaminated apple juice, and breakthrough capacities increased with temperature. The composite carbon adsorbent offered a higher initial breakthrough capacity than pelleted activated carbon. However, the appearance and taste of apple juice may be affected by the treatment process.

Efficient chemical decontamination strategies for patulin-contaminated foods like apple juice and apple juice concentrate do not exist. In the presence of sulfhydryl groups or sulfite, patulin is degraded rapidly (Aytac

and Acar, 1994; Fliege and Metzler, 2000). As reviewed by Steiner *et al.* (1999) at acidic pH, a reversible binding of sulfite to patulin occurs. The resulting hydroxysulfonate still includes the conjugated lactone ring, which is the toxicologically relevant structure. Furthermore, because of its allergenic potential, the use of sulfite in apple juice, which is frequently consumed by infants and young children, is not recommended.

As reported by Drusch *et al.* (2004), the presence of oxygen and free radicals is necessary for a rapid degradation of patulin by ascorbic acid. Oxidation of ascorbic acid in the presence of oxygen and metal ions is a possible source of these radicals. Because patulin degradation leveled off after complete oxidation of ascorbic acid, the initial concentration of ascorbic acid and its rate of degradation were important factors in patulin degradation. Because of the low oxygen content in the head space of a food package, addition of ascorbic acid to products like apple juice before filling is not an effective decontamination strategy. Furthermore, the toxicological potential of the resulting patulin degradation products remains unknown. Yazici and Velioglu (2002) investigated the effect of added thiamine hydrochloride, pyridoxine hydrochloride, and Ca-d-pantothenate at various doses to reduce the patulin content of apple juice concentrate. Addition of thiamine hydrochloride (1000 mg/kg), pyridoxine hydrochloride (625 or 875 mg/kg), and Ca-d-pantothenate (1000 or 2500 mg/kg), and storage at 4°C for six months yielded 55.5–67.7% of patulin reduction compared to only 35.8% for the control. Other quality parameters like clarity, color, and turbidity were not affected by this treatment.

Apart from patulin, *P. expansum* also may produce other mycotoxins like chaetoglobosin A and C, the communesins A and B, and the expansolides A and B. Larsen *et al.* (1998) described the production of all these mycotoxins by *P. expansum* when grown on black currant and cherry juice. In particular, chaetoglobosin A was found in juice when sufficient oxygen was available. These authors pointed out that this may pose a risk during juice production, particularly when juice tanks are only partly emptied and oxygen from the head space is available. Therefore, Larsen *et al.* (1998) recommend that tank head spaces may be supplied with nitrogen to control mycotoxin production during storage of juices. Andersen *et al.* (2004) also detected chaetoglobosins and communesins in naturally infected cherry and gooseberry juice samples. Because these samples did not contain patulin, a patulin-negative sample does not necessarily mean that the sample is free from fungal metabolites. These authors suggested that chaetoglobosin A may be a better indicator of growth of *P. expansum* in fruit products than patulin, and that in order to increase safety of fruit products, a simultaneous method for determination of patulin and chaetoglobosin A should be developed.

Ochratoxin A is mainly found in grape juice (Belli *et al.*, 2002; Cerutti *et al.*, 1982; Delage *et al.*, 2003; Fritz, 1983; Majerus *et al.*, 2000; Zimmerli and Dick, 1996). The concentration in red grape juice is usually much higher than in white grape juice. Zimmerli and Dick (1996) found up to 289 ng of ochratoxin A/L in red grape juice compared to 5 ng/L in white grape juice. Majerus *et al.* (2000) analyzed 27 and 64 samples of white and red grape juice, respectively. The 50th percentile of ochratoxin A in the samples was 90 ng/L for white grape juice samples compared to 270 ng/L for red grape juice samples. With 5.3 μg/L, the maximum ochratoxin A concentration in red grape juice also was much higher than in white grape juice (1.3 μg/L). A possible explanation is that a prolonged enzymatic treatment at increased temperature is done during production of red grape juice to increase color yield. This processing step may favor fungal growth and ochratoxin A production. However, in 12% of red grape juice samples and in 22% of white grape juice samples, no ochratoxin A was detectable (Majerus *et al.*, 2000), and, therefore, possibilities for prevention of mycotoxin contamination already exist. Majerus *et al.* (2000) found that all samples of orange juice ($n = 30$) and apple juice ($n = 33$) were free from ochratoxin A.

Abdel-Sater *et al.* (2001) found aflatoxin B_1 and G_1 in apple juice–based drinks marketed in Egypt up to 20–30 μg/L, aflatoxin B_1 in guava juice (12 μg/L), but not in mango juice or grape juice– and peach juice–based drinks. Taking into account that apple juice–based drinks analyzed in the study of Abdel-Sater *et al.* (2001) contained only 20% of apple juice, the initial contamination with aflatoxins of the juice was up to 150 μg/L. Data like this can only be attributed to poor hygienic conditions of the raw material and/or the production facilities. Abdel-Sater *et al.* (2001) and Ragab (1999) also emphasized the necessity of introducing concepts of good manufacturing practices and HACCP in this context.

In the last few years, because of the development of reliable liquid chromatographic (LC) methods with diode array detection or MS detection for quantification of low levels of *Alternaria* toxins, the presence of alternariol and alternariol methyl ether in fruit juices has attracted attention. Delgado and Gómez (1998) detected alternariol and alternariol methyl ether natural contaminants in 16 of 32 samples of apple juice concentrate. Levels of alternariol ranged from 1.35 to 5.42 ng/ml, and alternariol methyl ether was present at concentrations of up to 1.71 ng/ml. Similar concentrations have been reported by Lau *et al.* (2003), who describe sensitive LC-MS and LC-MS-MS confirmatory procedures based on atmospheric pressure chemical ionization with negative ion detection for the determination of alternariol and alternariol methyl ether. The natural occurrence of alternariol in nine samples of apple juice and in single samples of some other clear fruit beverages like grape juice, cranberry nectar, raspberry juice, and prune

nectar at levels of up to 6 ng alternariol/ml were confirmed. *Alternaria* toxins were stable in fruit juice. Scott and Kanhere (2001) investigated the stability of alternariol, alternariol methyl ether, and altertoxin I in apple juice. Alternariol and alternariol methyl ether were stable after heating of juice for 20 min at 80°C or during storage at room temperature for 20 days, and no apparent loss was observed by these authors.

C. WINE AND OTHER ALCOHOLIC BEVERAGES

The most important mycotoxin in wine is ochratoxin A. Several studies on ochratoxin A concentration in wine are available (Battilani and Pietri, 2002; Burdaspal and Legarda, 1999; Delage *et al.*, 2003; Lau *et al.*, 2003; Majerus *et al.*, 2000; Markaki *et al.*, 2001; Pietri *et al.*, 2001; Soleas *et al.*, 2001; Stefanaki *et al.*, 2003; Tateo *et al.*, 2000; Visconti *et al.*, 1999; Zimmerli and Dick, 1996), which show large differences in the incidence and level of ochratoxin A contamination. Levels of ochratoxin A are higher in red wine than levels in rosé and white wine in all these studies. The most comprehensive review was published in 2002 by the European Commission (European Commission [Directorate-General Health and Consumer Protection], 2002a). The total number of samples was 1470, with 59% positive. The levels of contamination ranged from 3 ng/kg (Spain) to 15,600 ng/kg (Italy). The weighted European mean level was 357 ng/kg. The most recent data for ochratoxin A in red wine are summarized in Table VII.

Several authors observed an interrelation between ochratoxin A concentration and geographical region. Majerus *et al.* (2000) analyzed 41 samples of European white wine and 94 samples of European red wine. These authors classified results according to wine regions as defined by the European Common Market Organization for Wine following natural criteria like soil, climate, and topography and lumped the results to one northern region including Germany, northern France, and northern Italy and one southern region covering central and southern Italy, southern France, Greece, and Spain. With a 90% percentile value of 0.42 μg/L for white wine and 0.11 μg/L for red wine, samples from the southern region had a higher level of ochratoxin A than samples from the northern region with a 90% percentile value of less than 0.01 μg/L for white wine and 0.06 μg/L for red wine. As shown in Table VII, Pietri *et al.* (2001) reported a similar gradient for Italian red wine and for Italian white dessert wine from different regions of Italy. All authors emphasized that the warm climatic conditions in southern regions of Europe favor growth of *Aspergillus* species and, therefore, favor ochratoxin A formation in wines from these regions.

TABLE VII
CONCENTRATION OF OCHRATOXIN A IN RED WINE

Origin of the samples	n	Range (ng/L)	Mean (ng/L)	Median (ng/L)	Reference
Europe	91	<3–603	—	54	Burdaspal and Legarda, 1999
Southern Italy	27	<10–7630	—	895	Visconti *et al.*, 1999
Worldwide		<10–7000	—	172	Majerus *et al.*, 2000
Morocco	14	28–3240	—	650	Filali *et al.*, 2001
Mediterranean countries	31	<2–3400	—	—	Markaki *et al.*, 2001
Italy (total)	96	<1–3177	—	90	Pietri *et al.*, 2001
Northwest Italy	23	<1–79	—	2	
Northeast Italy	19	<1–227	—	90	
Central Italy	30	<1–1450	—	134	
Southern Italy	24	10–3177	—	1264	
Worldwide	580	<50–200	—	—	Soleas *et al.*, 2001
Greece	104	<50–2690	—	90	Stefanaki *et al.*, 2003
South Africa	9	180–390	246	—	Shepard *et al.*, 2003
South America	22	28–70	39	—	Rosa *et al.*, 2004

Zimmerli and Dick (1996) pointed out that apart from climatic differences, different practices in grape cultivation (e.g., use of pesticides or different cultivars, and wine making) may influence the ochratoxin A concentration in wine. The latter include time and condition of storage of harvested grapes, type of maceration, and time and temperature of fermentation.

The difference in wine making is also the reason for the differences in ochratoxin A concentration between white, rosé, and red wine. White grapes are immediately pressed after harvest, whereas red wine grapes are mashed and macerated to extract anthocyanins from the berry skins. Maceration lasts either for several days at elevated temperature or for several hours using pectolytic enzymes after heating of the must to 80°C.

All cited studies show that ochratoxin A contamination of wine is a result of mold infection in the field and subsequent processing conditions and, therefore, dispute the statement of Zimmerli and Dick (1996) that ochratoxin A in wine of southern European origin is formed only after the harvest of the grapes. Zimmerli and Dick (1996) assumed that climatic conditions in the south of Europe are less humid than in central Europe and are, therefore, less favorable for mold growth on the field. The authors suggested that ochratoxin A–producing molds growing in the barrels and processing of moldy fruits significantly caused ochratoxin A contamination in wine. They gave further evidence to their hypothesis by analyzing must and wine

samples, in which similar levels of ochratoxin A contamination were observed. In the presence of ethanol, ochratoxin A is rather stable. Zimmerli and Dick (1996) report a conversion of ochratoxin A to its ethyl ester ochratoxin C under acidic conditions in the presence of ethanol.

Fining of wine, a common winery practice, reduced ochratoxin A levels in wine. Fining involves the addition of an adsorptive compound to reduce levels of certain compounds like protein particles that would cloud the wine and phenolic compounds like tannins that could cause bitterness and astringency in wine. Dumeau and Trione (2000) assessed the ability of winemaking additives to remove ochratoxin A from red wines. Cellulose at 50 g/hL or inertized yeasts at 50 g/hL removed only small amounts of ochratoxin A (8 and 13%). Silica gel at 50 g/hL removed 30% of ochratoxin A. A 50% reduction in ochratoxin A concentration could be achieved with activated charcoal at 20 g/hL or silica gel in combination with gelatin. Castellari *et al.* (2001) showed that in particular activated carbon and potassium caseinate remove high amounts of ochratoxin A from red wine. Fining agents were used at levels of 10 and 150 g/hL. Because of low adsorption of total polyphenols, the activated carbon can be used at a dosage higher than 10 g/hL and might completely remove ochratoxin A from red wine.

Nevertheless, good manufacturing practice (GMP) is an important tool for preventing ochratoxin A contamination in wine. According to Soleas *et al.* (2001), a maximum level of 200 ng of ochratoxin A/L is easily achievable. To investigate the possible relation between quality of wine and ochratoxin A concentration, Tateo *et al.* (2000) analyzed Italian table wines in multicomponent packages. Ochratoxin A was detected in 97% of the samples, and the concentration was above 1000 ng/L in 52% of the samples. The authors stated that these levels are generally higher than those found in good quality–bottled wines.

Apart from ochratoxin A in wine, for alcoholic beverages, the occurrence of patulin in apple cider has occasionally been reported. The frequency of contamination and the detected concentrations of patulin are generally very low (Armentia *et al.*, 2000; Food Standards Agency, 2003b; Jackson *et al.*, 2003; Tangni *et al.*, 2003). Moss and Long (2002) studied the fate of patulin in the presence of *Saccharomyces cerevisiae,* a yeast commonly used for hard cider production. *S. cerevisiae* degraded patulin during active fermentative growth, but not when growing aerobically. Products of patulin degradation were more polar than patulin, and these authors identified E-ascladiol as one major metabolite of patulin. One possible explanation for the presence of patulin in cider after fermentation is an addition of patulin-contaminated apple juice to cider. The addition of apple juice to cider is a common practice for the production of sweet apple cider in European countries and South Africa (Tangni *et al.*, 2003).

D. MARMALADES AND JAM

Traditional marmalades and jams are made almost entirely from fruits and sugar. Additional ingredients may be gelling agents, starch syrup, and acids. Marmalades and jams are thickened by boiling to a final moisture content of 30% and a total sugar concentration of approximately 60%. Consequently, traditional marmalades and jams have a reduced water activity (a_w) of 0.8–0.9, which allows mold growth, but which may not be favorable for mycotoxin production. As previously described (Table III), for *Aspergillus* species the difference between minimum a_w value for mold growth (a_w 0.77–0.83) and mycotoxin formation (a_w 0.83–0.88) is rather small. In contrast, for *Penicillium* species, the minimum a_w value required for fungal growth is between 0.82 and 0.85, whereas mycotoxin production requires a minimum a_w value of 0.99. Conditions for mold growth and mycotoxin formation in marmalades and jams change, when the level of added sugar decreases, as is the case for diabetic products. The a_w value is much higher and products have to be stabilized using preservatives.

Lindroth *et al.* (1978) studied the effects of storage temperature and water activity on patulin production by *P. expansum* in black currant, blueberry, and strawberry jams over a storage period of six months. Reduction of storage temperature from 22 to 4°C decreased both hyphal growth and patulin production. When the water activity of stored jam was reduced by the addition of 20 and 44% sugar, toxin production in the jams was reduced to 0.08–10% of the maximum occurring in unsweetened jams, despite that the addition of sugar stimulated hyphal growth. Katsumata *et al.* (2002) investigated the effects of 17 spice oils to inhibit the growth of *P. expansum* on strawberry jam and found cassia, cinnamon, and clove oils to be effective. Cinnamic aldehyde and eugenol demonstrated high inhibitory effects on the growth of this mold. The combined use of two constituents revealed that the combination of cinnamic aldehyde and β-caryophyllene was highly effective for growth inhibition, although β-caryophyllene demonstrated only a weak effect when used alone. Patulin and citrinin were not detected in the jam on which *P. expansum* grew.

Ochratoxin A formation in apricot jam has been investigated by Ruhland *et al.* (1998). Apricot jam prepared conventionally was inoculated with ochratoxin A–producing *A. ochraceus* and *P. verrucosum.* Fungal growth was detected after two weeks and mycotoxin production after five weeks. Ochratoxin A contents reached up to 86.3 µg/kg for *A. ochraceus*—and 94.6 µg/kg for *P. verrucosum*–inoculated jams. Seven of twelve naturally contaminated jam samples contained 0.09–14.33 µg/kg ochratoxin A. In a market survey, Engel (2000) could not detect ochratoxin A at a concentration higher than 0.1 µg/kg in 42 commercial jam samples.

In summary, a frequent and continuous occurrence of mycotoxins in fruit products is limited to aflatoxins in figs, to ochratoxin A in dried vine fruits, grape juice, and wine, and to patulin in apple juice. Although in the fruit-producing countries due to climatic conditions, fungal growth and mycotoxin contamination are not unavoidable, the principles of good agricultural practices are key elements in the prevention of possible mycotoxin contamination as outlined in this review for aflatoxins in dried figs. Manual sorting of the fruits or sorting techniques like analysis of bright green-yellowish fluorescence help to reduce mycotoxin levels in the fruits before processing or consumption. Trimming damaged or rotted fruit before juice pressing is an effective tool for reducing mycotoxin levels in juices like apple juice. However, concerning direct consumption of fruits, it has to be kept in mind that mycotoxins may penetrate through parts of the fruit into the sound tissue or throughout the whole fruit. Analysis of patulin may not be sufficient for evaluation of the mycological status of apple juice and an additional analysis for chaetoglobosin A may be necessary. More data on the occurrence of chaetoglobosin A in apple juice should be collected to substantiate this recommendation. Removal of patulin from juices using physical adsorption techniques is not applicable, because the color and taste of the product are adversely affected.

Ochratoxin A is mainly associated with grapes and derived products. Red grape juice contains higher levels of the toxin, because a prolonged maceration and an enzymatic treatment are applied to increase color yield. Because ochratoxin A is stable in ethanol, wine also is frequently contaminated with ochratoxin A, although fining reduces ochratoxin A levels to a certain degree.

V. IMPACT ON HUMAN NUTRITION

Before an evaluation of the impact of a possible mycotoxin contamination in fruits on human health, the general procedure of risk assessment and the outcome thereof for mycotoxins by international agencies is briefly described. With the establishment of the World Trade Organization in 1995, standards and recommendations elaborated by the Codex Alimentarius Commission (CAC) reflect the international consensus for health and safety requirements (Moy, 1998). The CAC is an intergovernmental institution, which was founded in 1963 by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO).

Risk assessment for mycotoxins is a multistep process that involves the CAC and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The CCFAC is responsible for the subsequent step, risk

management. Another important institution is the International Agency for Research on Cancer (IARC) of the WHO, which provides scientific backgrounds and evaluations concerning the carcinogenicity of chemical substances. Table VIII gives an overview on risk assessment for aflatoxins, ochratoxin A, and patulin.

Apart from hazard identification and characterization, a vital step in risk assessment is the exposure assessment for a population and the contribution of different food groups to the total intake. For ochratoxin A, a reevaluation has been performed by the JECFA (2001a). As stated by the JECFA, 85% of newly available data on ochratoxin A originated from Europe. This is probably because the European Union has established maximum levels for ochratoxin A for different commodities and research was focused on ochratoxin A in those products for years. Assessments of international ochratoxin A intake have been made by the JECFA on the basis of data on mean consumption combined with the weighted mean level of contamination. Because ochratoxin A occurs mainly in the diet of European countries, data on food consumption in Europe obtained from the Global Environmental Monitoring System/Food Contamination Monitoring (GEMS/Food) were considered the most relevant for risk assessment in the evaluation of JECFA (2001a). The submitted data on levels of contamination were aggregated according to the recommendations of a FAO/WHO workshop to obtain a weighted mean. Using this approach, the mean total intake of ochratoxin A was estimated as 45 ng/kg of body weight/wk assuming a body weight of 60 kg. Wine contributed about 10 ng/kg of body weight/wk to the mean intake (22%), whereas grape juice contributed 2–3 ng/kg of body weight/wk (6.7%). Dried fruits contributed less than 1 ng/kg of body weight/wk (JECFA, 2001a).

The situation concerning the contribution of fruits and derived products to the total ochratoxin A intake may change if either the basis for food consumption data or ochratoxin A concentration is changed. This becomes evident from data of a nationwide ochratoxin A survey performed in Germany (Cholmakov-Bodechtel *et al.*, 2000). Intake estimation of Cholmakov-Bodechtel *et al.* (2000) was based on the analysis of 7000 food samples from the German market and a questionnaire on nutritional and consumption habits of 2500 persons. The mean total daily ochratoxin A intake of adults was 39.9 ng. Wine including champagne contributed 1.4% and foods from the category juices, table water, and other nonalcoholic beverages 6.6%. Based on data for the highest plausible portion size from the questionnaire and the 90th percentile of ochratoxin A concentration, calculated total daily ochratoxin A intake increased to 247.9 ng. Furthermore, the contribution of wine including champagne rose to 11.3%, and the contribution of foods from the category juices, table water, and other nonalcoholic beverages was 12.5%.

TABLE VIII

SUMMARY OF THE OUTCOME OF THE RISK ASSESSMENT BY THE INTERNATIONAL AGENCY FOR RESEARCH ON CANCER AND THE CODEX ALIMENTARIUS COMMISSION FOR AFLATOXINS, OCHRATOXIN A, AND PATULIN IN FOODS FOR HUMAN CONSUMPTION[a]

	International Agency for Research on Cancer degree of evidence of carcinogenicity			
	Humans	Animals	Overall for humans	Codex Alimentarius Commission
Aflatoxins	Strong	Strong	Class 1 (carcinogenic to humans)	No tolerable intake defined; presence of aflatoxins in food should be reduced to irreducible levels[b]
Aflatoxin B_1	Strong	Strong		
Aflatoxin B_2		Limited		
Aflatoxin G_1		Strong		
Aflatoxin G_2		Inadequate		
Ochratoxin A	Inadequate	Strong	Class 2B (possibly carcinogenic to humans)	Provisional tolerable weekly intake: 0.1 μg/kg of body weight
Patulin	Inadequate; no data available	Inadequate	Class 3 (not classifiable as to its carcinogenicity to humans)	Provisional tolerable daily intake: 0.4 μg/kg body weight

[a]Codex Alimentarius Commission, 2002; International Agency for Research on Cancer, 1993; Joint Expert Committee on Food Additives (JECFA), 1998; Joint Expert Committee on Food Additives, 2001b.

[b]According to the JECFA, the irreducible level is defined as the concentration of a substance that cannot be eliminated from a food without involving the discarding of that food altogether, severely compromising the ultimate availability of major food supplies. Also referred to as the *ALARA principle* ("as low as reasonably achievable").

Another crucial point is that the contribution of fruits and derived products contaminated with ochratoxin A still may contribute significantly to the total ochratoxin A intake of individual groups within a population, which may possess a dietary pattern different from the average adult consumer. Within a study on the Europe-wide assessment of ochratoxin A intake (European Commission [Directorate-General Health and Consumer Protection], 2002a), the United Kingdom provided data for ochratoxin A intake by children aged 1.5–4.5 years. Total dietary ochratoxin A intake of the children was 3.55 ng/kg body weight/day. With 2.2 ng/kg body weight/day, dried fruits contributed 62% to total dietary ochratoxin A intake.

The situation is similar for patulin. Based on available risk analysis studies, dietary intake of patulin by the general population is not supposed to be a health risk, but again, infants and young children as heavy consumers of possibly contaminated apple products may be exposed to higher levels of patulin. The U.S. Food and Drug Administration (FDA) published in 2001 a background paper on patulin in apple juice, apple juice concentrates, and apple juice products, in which dietary intake of patulin was assessed (FDA, 2001). In this study, the FDA considered the estimated exposure to patulin for drinkers of "all ages" and for small children in two age categories, children younger than 1 year and children 1–2 years old using a probabilistic modeling, the so-called "Monte Carlo analysis." Two scenarios were calculated, one on the basis of no control measures of producers in which all available data on patulin in apple products were taken into account. In the second scenario, on the basis of a maximum patulin level of 50 μg/kg, producers do not place products with patulin levels higher than 50 μg/L on the market. The results of the assessment indicated that if no controls for patulin levels are carried out by producers, the estimated 90% percentile patulin exposure for apple juice drinkers of all ages was approximately 0.26 μg/kg of body weight/day. The exposure for children younger than 1 year was 0.4 μg/kg of body weight/day and exposure for children 1–2 years was 1.7 μg/kg of body weight/day, four times the provisional tolerable daily intake. Furthermore, the results showed that if processors implement controls for patulin at the 50-μg/kg level, the estimated 90% percentile decreased to 0.1 μg/kg of body weight/day for drinkers of all ages, to 0.27 μg/kg of body weight/day for the younger than 1-year-old age-group and to 0.67 μg/kg of body weight/day for 1- to 2-year-old children. Based on these data, the FDA recommended a 50-μg/kg action level for patulin in apple juice, apple juice concentrates, and apple juice products (FDA, 2001).

An assessment of the dietary exposure to patulin in Europe showed that as far as the comparison with the provisional tolerable daily intake of 0.4 μg/kg of body weight/day is concerned, from the data reported, the exposure seems to be quite below that value (European Commission

[Directorate-General Health and Consumer Protection], 2002b). Apple juice and apple nectar represented the main source of intake in Austria, Belgium, France, Germany, Portugal, and the United Kingdom for all groups of population taken into consideration, particularly for young children. Cider, including drinks based on cider, provided a considerable contribution to the total intake of consumers in France; for male adults in France it was the main contribution.

Concerning the contribution of fruits, dried fruits, and fruit-containing products to the total dietary intake of aflatoxins, no data are available. The latest risk analysis on aflatoxins was performed by the JECFA in 1998. The JECFA stated that although a wide range of foods may be contaminated with aflatoxins, they have been most commonly associated with groundnuts, dried fruits, tree nuts, spices, figs, crude vegetable oils, cocoa beans, maize, rice, cottonseed, and copra. Because only few data on dried fruits were available, the JECFA intake estimation for aflatoxin B_1, B_2, G_1, and G_2 is almost entirely based on aflatoxin contamination data and intake data for cereals and nuts. However, heavily aflatoxin-contaminated dried fruits, especially figs, may pose a health risk for consumers and maximum levels as set in the European Union, and effective control measures are unavoidable. In 2002, special conditions on the import of figs, hazelnuts, pistachios, and certain products derived thereof originating in a specific third country had to be established by the European Union. Maximum limits had been considerably exceeded several times, and after carrying out a mission to the country, the European Commission's Food and Veterinary Office (FVO) stated "that the control procedures in place for dried fig consignments intended for export into the European Community do not ensure that the consignments comply with the maximum levels established in EC legislation. Insufficient training of responsible officials, insufficient sampling and testing procedures and insufficient evidence that the export certificates correlate to the concerned consignment have been observed" (European Commission, 2002).

As discussed, aflatoxins are genotoxic and carcinogenic, and for substances of this type, there is no threshold below which no harmful effect is observed. Subsequently, no tolerable daily intake has been set by the JECFA or any other international or national authority. Limits for foods are set as low as reasonably achievable. The current legislation in the European Union includes a maximum level of 2 μg/kg for aflatoxin B_1 and 4 μg/kg aflatoxin B_1, B_2, G_1, and G_2 for dried fruits and products processed thereof, intended for direct human consumption or as an ingredient in foodstuffs.

In conclusion, the risk of an acute toxicosis or long-term chronic health effects of a mycotoxin contamination through consumption of mycotoxin-contaminated fruits and fruit products is relatively low compared to other food groups such as cereals. The occurrence of an endemic poisoning by

consumption of mycotoxin-contaminated fruits has so far not been reported in contrast to mycotoxin poisonings by consumption of cereals. Nevertheless, regional problems may arise in tropical or subtropical areas, where climatic conditions favor mold growth and mycotoxin formation, where food consumption pattern may be unvaried, and where hygienic conditions during storage and processing may be poor.

Certain groups of a population may be at risk for elevated exposure to mycotoxins if certain fruit products significantly contribute or even dominate the daily diet. This has especially been shown for infants and young children for patulin in apple juice and ochratoxin A in dried vine fruits. Furthermore, wine and cider may significantly contribute to ochratoxin A intake of adults. As a consequence, maximum mycotoxin concentrations for certain fruit products have been established in several countries.

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