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Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of Food Safety Objectives

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ABSTRACT

The concept of Food Safety Objective (FSO) has mostly been applied to understanding the effects of handling and processing on levels of bacterial pathogens in foods, but it is also applicable to the formation and removal of mycotoxins. This paper provides a general overview of how the concept of FSO can be used to understand increases and decreases in mycotoxin levels in foods, on the basis that international regulatory limits are equivalent to an FSO. Detailed information is provided on the ecology of the formation of aflatoxins, fumonisins, ochratoxin A and deoxynivalenol in major commodities. Methods in use to reduce levels of these mycotoxins, to meet an FSO, are then detailed. Each of the major mycotoxin – food combinations is visualised using a novel graphical method.

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1. Introduction

Regulatory efforts internationally have focused on the use of risk assessment tools to drive food safety policy and standards away from prescriptive to outcomes based on concepts such as the Food Safety Objective (FSO) and Performance Objectives (CAC, 2007; ICMSF, 2002a). These approaches provides a scientific basis that promotes flexibility and innovation by allowing industry to select and implement control measures specific to particular operations. Many current food safety issues are complex in nature, requiring approaches through the production chain and relying on more than one control measure to manage risk effectively. It is envisaged by regulators around the world that the new risk management guidelines will offer a framework that will facilitate communication between stakeholders on the most effective food safety management options as well as providing a scientific basis for equivalency.

The risk management framework approach has seen wide application in the development of Codex Alimentarius codes for the control of *Listeria* in ready to eat products and within the hygienic code of practice for powdered infant formula. More recently, this framework has been used as the basis for the validation of control measures in a food chain and in the consideration of alternative measures to ensure the safety of commercially sterile foods (Anderson et al., 2011).

The FSO concept has generally been applied to issues regarding safety from pathogenic and toxigenic bacteria, but has wider application, for example in regard to the formation and control of mycotoxins. Theoretical aspects of this topic have recently been reviewed by García-Cela, Ramos, Sanchis, and Marin (2012). In the current paper, the ICMSF/CODEX risk management framework is used as a tool to assist in explaining the ecology of mycotoxin formation in major food commodities and to highlight the control measures available to manage mycotoxin levels in foods, to meet Food Safety Objectives.

The toxicity of important mycotoxins has been evaluated by international specialists, most notably by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA) and the US National Toxicology Program (NTP). In particular, JECFA provides estimates of toxicity to Codex, which determines levels of mycotoxins permissible in foods and food commodities in international trade. Although explicitly stated only rarely, i.e. in ICMSF (2002b) and García-Cela et al. (2012), such maximum permitted levels possess essentially the same status as FSOs determined for bacteria in foods.

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In the case of bacteria, the general formula

$$H_o - \sum R + \sum I = FSO$$

is relevant, as reductions in numbers of bacteria result from some form of processing such as heating, and increases in numbers may occur subsequently (ICMSF, 2002a). In the case of mycotoxins, the formula is more logically used in the reverse order

$$H_o + \sum I - \sum R = FSO$$

as increases in mycotoxin levels may occur before or after harvest, during drying, or during storage ($\sum$I). Reduction in mycotoxin levels, $\sum$R, takes place during processing (ICMSF, 2002b).

1.1. Assumptions and caveats

The time when "H_o", the initial level of contamination, occurs during mycotoxin formation is debatable. Some logic exists in placing H_o at the time when edible portions of crops begin to develop, or begin to mature. However, those points are at best uncertain, i.e. mycotoxin levels are not usually analysed then, and levels are almost always uncontrolled. Drying and storage may take place on farm, and some merit exists in placing H_o at the time of harvest. However, these steps rarely result in any decrease in mycotoxin levels (except for cleaning, a process neglected here). For the sake of simplicity, for the purposes of this work, H_o is designated as the time of sale from the farm to distributors or processors, following which mycotoxin reduction usually takes place. For present purposes, drying and storage on farm is not differentiated from later drying and storage, as the effects of poor drying and storage on farm or in warehouse or factory, or in transport, are similar.

The approach taken here is entirely qualitative, i.e. no weight is given to slopes of lines in the figures, so all have been drawn at the same angle. In practice, increases or decreases in mycotoxin levels in any commodity are strongly dependent on climate, storage and processing conditions. Any quantitative risk management framework for a particular situation would require the appropriate data to allow estimation of stochastic aspects at each stage. A similar approach to that of Zwietering, Stewart, Whiting, and International Commission on Microbiological Specifications for Foods (2010) would be required. Climatic modelling has been shown to assist in managing aflatoxin in Australian peanuts (Chauhan et al., 2010) and deoxynivalenol in Canadian wheat (Schaafsma & Hooker, 2007). In the same way, no figures are given for FSOs, as the focus of this paper is the conveyance of the concept of risk management to the issue of mycocotin control, not quantifying acceptable levels of protection.

It is recognised that the following discussion relates to what is believed to be normal commercial practice. Under exceptional circumstances, mycotoxins may form at different times, or different reduction strategies may apply. It is impractical to attempt to accommodate all such possibilities in a general paper of this type.

1.2. Mycotoxins

According to Miller (1995) five mycotoxin groups are of importance in human health: aflatoxins, ochratoxin A, fumonisins, trichothecenes, specifically deoxynivalenol and closely related compounds, and zearalenone. These will be treated here, with the exception of zearalenone, as it is produced by the same fungi as produce deoxynivalenol, so production and removal follow similar pathways.

2. Aflatoxins

Aflatoxins are produced by a number of species of *Aspergillus*, of which *Aspergillus flavus* and *Aspergillus parasiticus* are the most important in foods. *A. flavus* produces B aflatoxins, while *A. parasiticus* produces both B and G forms. While only 40% of *A. flavus* isolates produce aflatoxins in culture, essentially all *A. parasiticus* strains are producers. The most important commodities affected by these species are peanuts, maize and, in the USA, cottonseed. Although *A. flavus* infects all of these crops, *A. parasiticus* is usually only associated with peanuts. Aflatoxins occur to a lesser extent in many other crops, including tree nuts, spices, rice, etc (Pitt & Hocking, 2009). Aflatoxins are perhaps unique among mycotoxins, as they are produced both before and after harvest under conditions that occur quite commonly.

Aflatoxins are the most important mycotoxins, as aflatoxin B_1 is the most potent liver carcinogen known. It is likely that aflatoxins produce other effects in humans as well (Khlangwiset, Shephard, & Wu, 2011; Williams et al., 2004).

2.1. Aflatoxins in peanuts

Peanuts are unique among nut crops, as the nuts develop underground, conditions favourable for attack by both insects and fungi. The time course of aflatoxin development and reduction in peanuts in good commercial practice is shown in Fig. 1.

2.1.1. Preharvest

Under conditions of adequate rainfall or irrigation, aflatoxin usually does not occur in peanuts. However, much of the world's peanut crop is produced under less than ideal conditions. Peanut plants have deep tap roots and so have more resistance to drought than many other crops. For this reason, peanuts are often grown under moisture limiting conditions, and in the tropics that often means towards the end of the rainy season, after rice or some other more drought sensitive crop. The major factors influencing *A. flavus* and *A. parasiticus* infection in peanuts are insect damage to the developing nuts and plant stress due to drought and high soil temperatures before harvest (Dorner, Cole, & Blankenship, 1998; Pitt, 2004). Although it is known that developing peanuts can be infected by a variety of means, including through flowers or systemically, most infection takes place directly from the soil surrounding the nut. Insect damage provides direct access through the shell. Drought stress acts in three ways: first, by reducing the plant's natural defences against infection (well developed in a nut that forms underground) as the plant wilts and loses metabolic activity; second, by reducing the water activity in the soil, which reduces growth and activity of bacteria, amoebae and competing fungi; and third, by promoting growth of *A. flavus* and *A. parasiticus*, which are xerophiles (Pitt & Hocking, 2009).

Reductions in drought stress by irrigation, or rain, limiting insect damage by good agricultural practice, or competitive exclusion by introduced nontoxigenic strains of *A. flavus* (biocontrol; Dorner, Cole, & Blankenship, 1992; Pitt, 2004), all assist in reducing the occurrence of aflatoxins before harvest. However, drought stress cannot be prevented under the dry culture condition under which most of the world peanut crop is produced. In much of the world, good agricultural practice cannot prevent aflatoxin production in peanuts before harvest.

2.1.2. Postharvest

As with other crops, rapid drying of peanuts will prevent any increase in aflatoxin production. This requires mechanical systems. The usual practice in industrialised economies is to pull bushes from the soil and invert them on the row to permit sun drying.

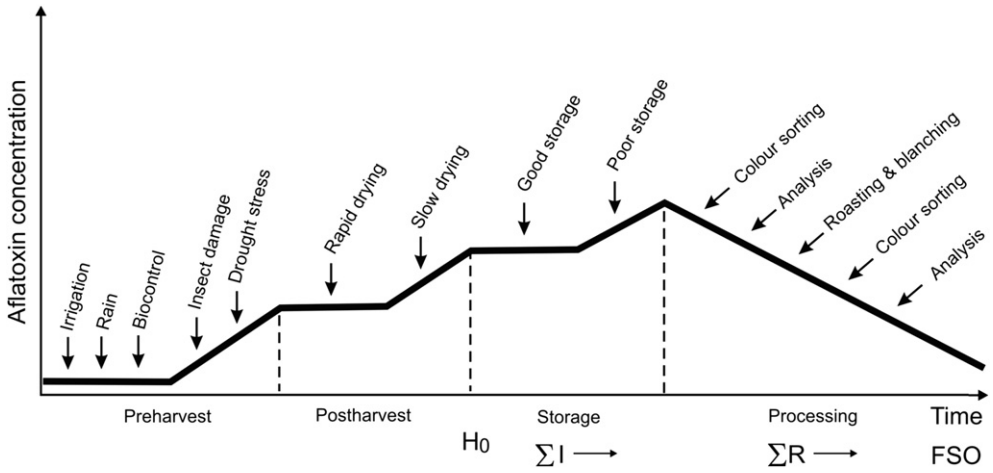

Fig. 1. The time course of aflatoxin formation and reduction in peanuts, with reference to the Food Safety Objective.

However, this process, which takes 6–10 days (Pitt, 1989) is not rapid enough to prevent aflatoxin increase. Stripping peanuts from the bushes before drying, a common practice in Southeast Asia, is probably preferable. A more effective technique is mechanical harvesting of freshly dug peanuts, followed by gentle mechanical drying. This is practised in some Australian areas. However, most peanut crops are sun dried, and limiting aflatoxin production depends on the vagaries of the weather.

2.1.3. Storage

If peanuts are dried effectively and kept dry in well designed silos where moisture migration does not occur, or are stored under refrigeration below 10 °C, aflatoxin concentrations do not increase. However, many peanut growing and storage areas are tropical, with high humidities and less than perfect storage facilities. As *A. flavus* and *A. parasiticus* are xerophiles, aflatoxin production will continue to occur if storage floors are damp, or humidities rise significantly above 80% RH. Poor storage may result in a positive value for $\sum$I.

2.1.4. Processing

Peanuts cannot be sorted by fluorescence, as they fluoresce inherently. However, advantage is taken of the fact that fungal growth of any sort usually results in nut discolouration. After shelling, sorting of individual kernels by hand, or preferably by machine, can remove discoloured nuts, and a very high proportion of aflatoxins also. The aflatoxin level is checked by careful sampling, preferably on line, and analyses. If the FSO has not been attained, lots may be blanched to remove skins and roasted, which increases discolouration, then colour sorted again. Further sampling and analysis will result in attaining the FSO.

In less industrialised economies, hand sorting may not be adequate. In subsistence economies, peanuts are usually eaten at source, where no checking or even sorting may take place. There the internationally recognised maximum acceptable levels are only infrequently attained (Pitt & Hocking, 1996). Some processing techniques available in village economies can reduce aflatoxins in peanuts, especially dry roasting (Njapau, Muzungaile, & Changa, 1998).

2.2. Aflatoxins in maize

Maize is grown in nearly all warm temperate and tropical zones, with high yields of both kernels and animal fodder. Maize has a much shallower rooting system that peanuts, increasing the risk of drought stress. The formation of kernels in cobs in the air renders maize liable to attack by a variety of airborne insects.

2.2.1. Preharvest

The pattern of development of aflatoxins in maize follows a similar course to that in peanuts, but with important differences (Fig. 2). As with peanuts, preharvest increases in aflatoxins are due to drought stress and insect damage, though in maize insect damage is probably more severe than in peanuts. The use of Bt maize cultivars can significantly decrease aflatoxin production by reducing insect damage (Dowd, 2001).

2.2.2. Postharvest

Rapid drying is also important in maintaining H_o at low levels. Where maize matures under dry conditions, as in much of Africa, cobs are usually left in the field to dry on the stalk, and postharvest aflatoxin increase is minimal. However, in Southeast and East Asia, maize is frequently harvested wet, to take advantage of residual soil moisture to plant a second crop before the onset of the dry season. In Southeast Asia, maize may be piled in stacks in the field to dry, which takes time, or may be shelled wet and then dried, which again may result in aflatoxin increases before the time of H_o (Siriacha, 1991).

In northern China, shelled maize may be left frozen, then thawed and dried during winter, limiting aflatoxin production (Borompichaichartkul, Srzednicki, & Driscoll, 2003).

2.2.3. Storage

Storage in well constructed silos to prevent moisture migration will limit aflatoxin production. However, storage in less developed economies is often less satisfactory, in uninsulated metal silos subject to moisture migration, buildings with leaky roofs, or earthen floors, or in outdoor wooden bins. Increases in aflatoxin, $\sum$I, frequently occur.

2.2.4. Processing

In temperate zones such as the United States, commercial practice is to screen samples of cracked kernels from large lots under UV light, where bright yellow green fluorescence indicates the presence of aflatoxin. Suspect lots are then assayed for aflatoxins by more precise methods, and those over accepted limits diverted to animal feeds. This technique is of no value in the tropics, as kojic acid, the compound on which fluorescence depends, isomerises at temperatures above 30 °C.

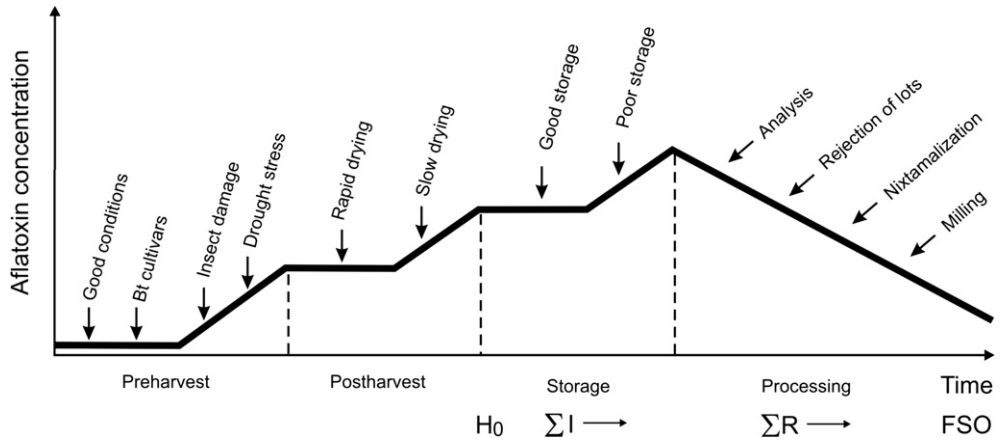

Fig. 2. The time course of aflatoxin formation and reduction in maize, with reference to the Food Safety Objective.

Sorting of individual kernels, as used for peanuts, is not normally attempted on maize lots, although modern machinery may be capable of such sorting.

In Central America, the process known as nixtamalization is commonly used in the preparation of maize meals to make tortillas and similar foods. This process destroys aflatoxins, and provides an effective means of meeting the FSO (De la Campa, Miller, & Hendricks, 2004). Otherwise, rejection of maize lots with excessive aflatoxin is the only means normally used to control aflatoxin levels.

2.3. Aflatoxin in brazil nuts

2.3.1. Preharvest

Brazil nuts grow on tall forest trees (*Bertholletia exelsa*) in the Amazon basin. They are difficult to cultivate, and almost all commercial nuts are still collected and sold by indigenous people. The nuts are formed inside pods the size of small coconuts, up to 40 per pod. When mature the pods fall to the forest floor, from whence they are collected at more or less frequent intervals. Pods may lie on the forest floor for several days to several weeks before collection due to climatic conditions and collection methods. No evidence has been found that infection by *A. flavus* occurs on the tree, but seems to take place before collection, by entry through the pod from soil on the forest floor. *Aspergillus nomius* has also been shown to be a common source of aflatoxin in brazil nuts (Olsen, Johnsson, Moller, Paladino, & Lindblad, 2008). Some less commonly occurring *Aspergillus* species have also been found in brazil nuts (Calderari et al., in press), but appear unimportant as sources of aflatoxin contamination.

2.3.2. Postharvest

Pods collected from the forest floor are taken to centres in the forest, where common practice is to open the pods. Nuts, still in shell, are dried in the sun or transported to processors for drying. H_o is therefore variable, and depends on the length of time before collection of the pods, and the time and rate of drying (Fig. 3).

2.3.3. Storage

Storage time in the forest depends on season and price. In due course nuts are transported along the Amazon or its tributaries to markets, mainly in Manaus and Belem. Nuts may be sold locally without inspection or analyses, or may be sold to processors. Processors may store the nuts in shell in warehouses, of varying quality. Aflatoxins may increase during transport and storage, leading to a positive $\sum I$.

2.3.4. Processing

Nuts are shelled by hand in factories employing some hundreds of people. Visual inspection removes nuts that are broken, show decay or discolouration. Size grading, gravity sorting and aflatoxin analyses are carried out for export or some internal markets (CAC,

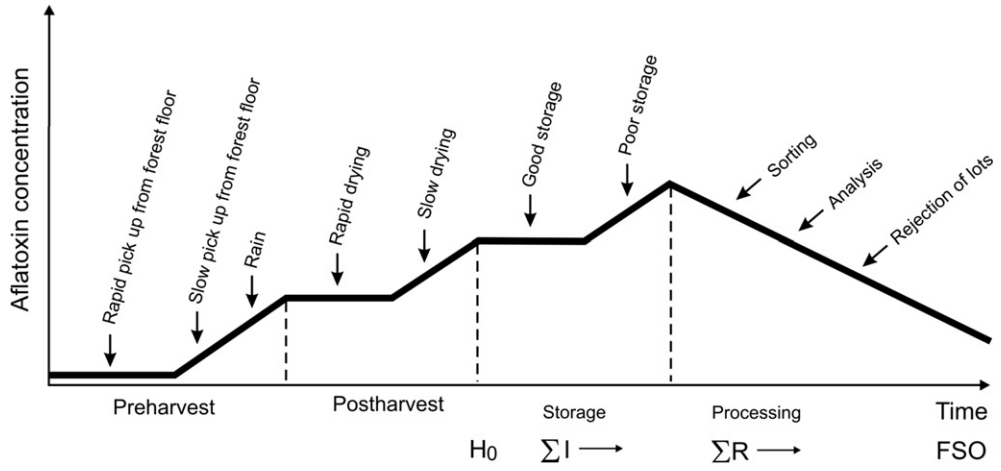

Fig. 3. The time course of aflatoxin formation and reduction in brazil nuts, with reference to the Food Safety Objective.

2010). Some problems still exist in satisfactorily meeting the FSO. As the nuts are large, visual inspection by consumers can reduce consumption of nuts containing unacceptable levels of aflatoxin (Marklinder, Lindblad, Gidlund, & Olsen, 2005).

2.4. Aflatoxin in other tree nuts

Tree nuts are encased in hard shells, limiting infection by *A. flavus*, so levels of aflatoxins are usually low in relation to levels in maize or peanuts. However, in almonds and pistachios aflatoxin levels may be significant. Insect damage preharvest is the most important problem for almonds (Campbell, Molyneux, & Schatzki, 2003; Schatzki & Ong, 2000, 2001)(Fig. 4). As with other commodities, rapid drying and good storage are important in limiting $\sum$I. Sorting, analysis and rejection of lots with excessive aflatoxin are standard procedures in California, where most of the world's almonds are grown.

Pistachio nuts can also be damaged by insects with consequent aflatoxin production (Michailides, 1989) (Fig. 5). However, the more important problem preharvest is that pistachios have been bred to have hulls that split open around harvest, for ease of consumption. However, some cultivars produce hulls that split early, before the kernels dry appreciably, allowing entry of *A. flavus* (Doster & Michailides, 1994, 1995; Sommer, Buchanan, & Fortlage, 1986).

After harvest, flotation or colour sorting of shelled nuts can be effective (Schatzki & Pan, 1996; Takahashi, Okana, & Ichinoe, 2001), but pistachios are usually sold in hull. Roasting causes some reduction in aflatoxin levels, but effective treatments may cause flavour damage (García-Cela et al., 2012; Yazdanpanah, Mohammadi, Abouhossain, & Cheraghali, 2005). UV light sorting is ineffective for pistachios, as the nuts fluoresce. Conventional colour sorting is of limited use, but more sophisticated imaging systems have been suggested (Pearson, Doster, & Michailides, 2001).

2.5. Aflatoxins in small grains: wheat, barley and rice

Few reliable reports have been published of significant levels of aflatoxins in small grains. The principal reason appears to be that *A. flavus* has no affinity with small grain cereal plants, members of the grass family *Poaceae*, and does not invade these plants before harvest. Perhaps the plants also have defence mechanisms. In addition, barley grows in cool, damp climates unsuited to *A. flavus*. Rice is grown under water in the early stages of development, so levels of *A. flavus* in rice growing soils are very low. In addition, the process of hulling rice creates heat, so freshly bagged, hulled rice has a very low fungal load. Of 42 samples of paddy and hulled rice from Thailand, only 10 had any infection with *A. flavus*, and the level of infection in individual grains within those samples did not exceed 4% (Pitt et al., 1994).

It seems likely that if these grains mature as standing crops, where levels of *A. flavus* are low, little infection takes place and aflatoxin levels are reliably low. If these crops are harvested wet and then dried, infection by *A. flavus* and aflatoxin formation become more likely (Fig. 6). The little available information suggests that if small grains do contain unacceptable levels of aflatoxin, this is more likely to result from poor storage. Most grain producing and importing countries do not routinely analyse these commodities for aflatoxins, so no reduction step normally is applied (Fig. 6). Note that the shape of Fig. 6 does not imply that small grains frequently do not meet any designated FSO, merely that no processing steps to reduce aflatoxins are normally applied, or indeed necessary.

A survey in Canada of 200 imported rice samples from two years showed mean aflatoxin concentrations of less than 0.2 μg/kg; of the five most contaminated samples in each year, only one exceeded 3.5 μg/kg aflatoxin B_1 (Bansal et al., 2011).

3. Fumonisins

Fumonisins are produced by a small number of *Fusarium* species, particularly *Fusarium verticillioides* and the less frequently isolated but related species *Fusarium proliferatum*. The habitat for these fungi is maize and fumonisin production by *Fusarium* species in other crops is uncommon (Pitt & Hocking, 2009).

It has recently been shown that fumonisins are also produced, quite unexpectedly, by *Aspergillus niger*. Studies on the genome sequence of *A. niger* showed the presence of the genes for fumonisin (Baker, 2006). It was soon confirmed that this gene cluster was active, and that many strains of *A. niger* produce fumonisins (Frisvad, Smedsgaard, Samson, Larsen, & Thrane, 2007). The significance of this to human health is still unclear, but *A. niger* is of common occurrence, so that the number of commodities that may contain fumonisins has greatly expanded recently. Fumonisin production by *A. niger* will not be dealt with here, but the ecology of the fungus is addressed under ochratoxin A (Section 5).

3.1. Fumonisins in maize

3.1.1. Preharvest

F. verticillioides is endemic in maize and occurs wherever maize is grown. Under good growing conditions it is a commensal (not unlike *A. flavus*), causes little damage to kernels and little fumonisin

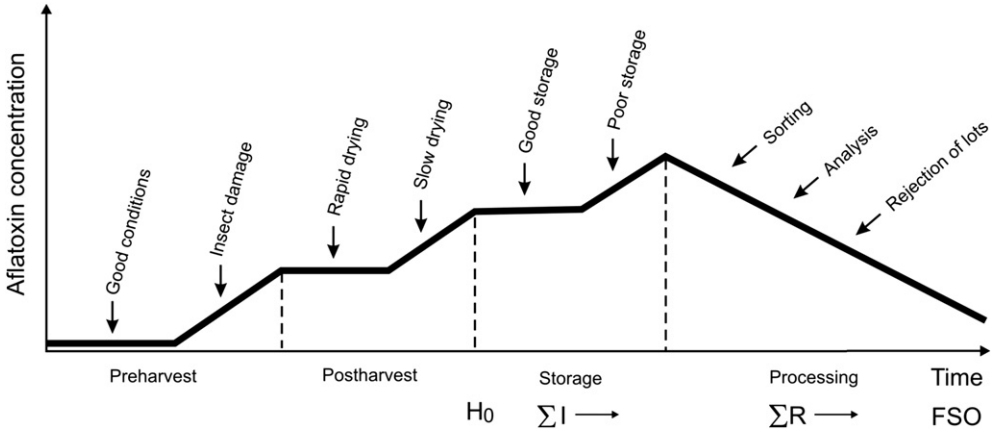

Fig. 4. The time course of aflatoxin formation and reduction in tree nuts other than brazil nuts and pistachios, with reference to the Food Safety Objective.

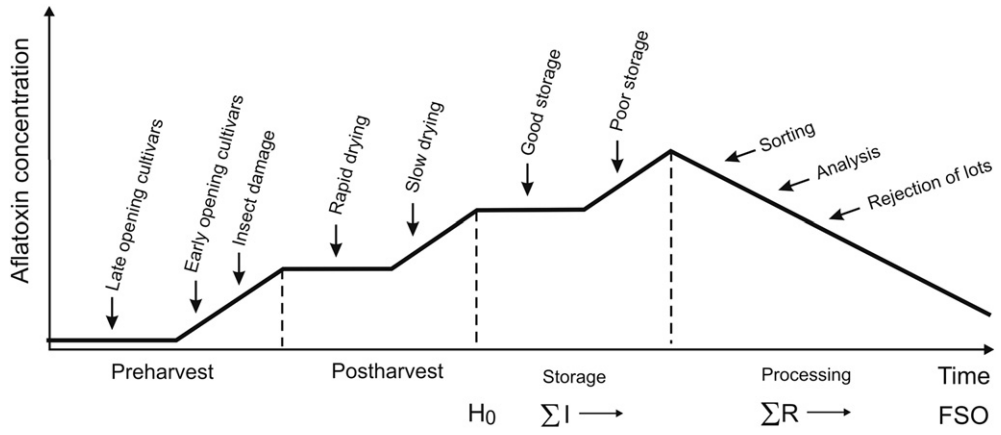

Fig. 5. The time course of aflatoxin formation and reduction in pistachio nuts, with reference to the Food Safety Objective.

formation. However, drought stress and insect damage cause a great increase in growth of the fungus, and hence in fumonisin production (Fig. 7). Rain, irrigation and growth of Bt maize cultivars are all important factors in limiting fumonisin production. Bt maize cultivars inhibit the proliferation of Lepidopteran insects, the main invaders of maize kernels in many places (Bakan, Melcion, Richard-Molard, & Cahagnier, 2002; Munkvold, Hellmich, & Rice, 1999; Munkvold, Hellmich, & Showers, 1997). An additional factor is that the use of cultivars developed for particular climates is important: use of hybrid strains outside the recommended areas increases stress and fumonisin production (Doko, Rapior, Visconti, & Schjoth, 1995; Visconti, 1996).

3.1.2. Postharvest

As with the formation of aflatoxins, rapid drying is recommended practice, but is of importance in this case only in the initial stages of drying. *Fusarium* species do not grow below about 0.9 water activity (a_w), so once the kernel moisture content has been reduced below that figure, fumonisin accumulation ceases. This frequently occurs in field drying before harvest of the cobs.

3.1.3. Storage

As noted above, *Fusarium* species grow very little below 0.9 a_w, so fumonisins will not be produced in storage. Under all normal conditions, $\sum I = 0$. Even if very high moisture occurs due to water ingress, competition with other microorganisms at such high water activities will prevent any significant increase in fumonisin levels.

3.1.4. Processing

In most geographical areas, the main methods for meeting the FSO are visual inspection of lots for fungal damage, fumonisin analyses, and rejection of lots that do not meet specifications. Milling of maize grains and separation of germ and bran substantially increases acceptance of maize flour (Pietri, Zanetti, & Bertuzzi, 2009).

Thermal processing below 150 °C has little effect on fumonisin concentrations, but extrusion, used extensively in the production of breakfast cereals and snack foods, substantially reduces fumonisin levels, especially in the presence of glucose (Bullerman & Bianchini, 2007; Bullerman et al., 2008; Jackson et al., 2011).

In Central America, the process of nixtamalization removes almost all fumonisins as well as aflatoxins, resulting in tortillas and other maize based foods being substantially free of these mycotoxins (De la Campa et al., 2004).

4. Deoxynivalenol and nivalenol

The major trichothecene mycotoxins produced in foods are deoxynivalenol (DON) and nivalenol (NIV). These compounds result from the growth of *Fusarium graminearum*, *Fusarium culmorum* and some related species, and they principally occur in small grains, especially wheat and barley. These species also produce the oestrogenic mycotoxin zearalenone. It occurs under similar conditions to DON, so it will not be treated separately here.

4.1. Deoxynivalenol in wheat

4.1.1. Preharvest

Unlike the other fungi of relevance here, *F. graminearum* and related species are true plant pathogens, being responsible for the disease in small grain crops known as Fusarium head blight. Unlike the toxins treated above, DON is not formed under drought stress, but as the result of rain. Increased rainfall promotes Fusarium head blight, with the incidence most affected by excessive moisture (rain

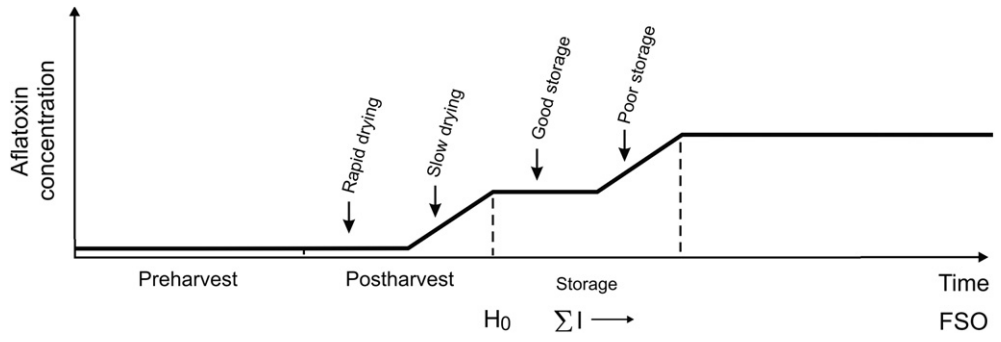

Fig. 6. The time course of aflatoxin formation in small grains, with reference to the Food Safety Objective.

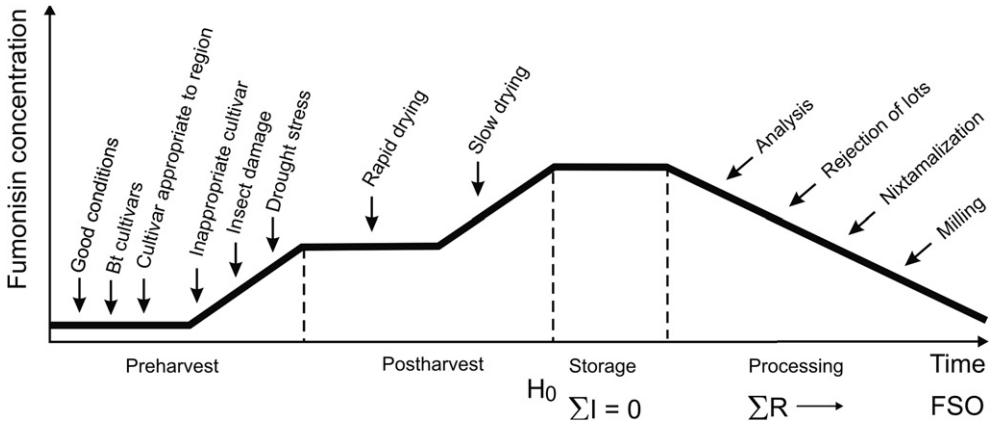

Fig. 7. The time course of fumonisin formation and reduction in maize, with reference to the Food Safety Objective.

or dew) at anthesis (flowering) (Fig. 8). Crop rotation is also important: growing wheat after maize increases Fusarium head blight under favourable weather conditions (Schaafsma, Tamburic-Ilincic, & Hooker, 2005). Ideal growing conditions for wheat require rain early in the growing season, and hot, dry finishing conditions. DON and NIV are uncommon in Australian wheat, but more likely to occur in the cooler, damper growing areas of much of the rest of the world. Insect damage does not appear to be an important factor in small grain infections preharvest (Miller, 1994).

4.1.2. Postharvest

Very high humidities (rain or mist) will cause increases in DON or NIV concentrations immediately after harvest. However, as *F. graminearum* and the related species are able to grow only at high water activities, DON and NIV production cease once the grain a_w drops below 0.9. The H_o values are usually the same as those present at harvest, unless damp weather is persistent.

4.1.3. Storage

The inability of *F. graminearum* and related species to grow below 0.9 a_w means that DON and NIV levels will not rise during storage. Here again $\sum I = 0$.

4.1.4. Processing

The primary method for meeting the FSO for DON or NIV is visual inspection of samples from lots, as *Fusarium* infection often causes grains to turn pink. Following that, suspect lots are usually analysed for the appropriate toxin, and diverted to animal feeds if they fail to meet the FSO.

Gravity sorting (Hazel & Patel, 2004) and optical sorting (Delwiche, Pearson, & Brabec, 2005) can reduce DON levels, but reported results are variable. Milling reduces DON or NIV levels, as much of the toxin is produced in the germ (Rios, Pinson-Gadais, Abecassis, Zakhia-Rozis, & Lullien-Pellerin, 2009; Thammawong et al., 2010). DON and NIV are relatively heat resistant; processes vary in effectiveness in reducing concentrations in baked products (Hazel & Patel, 2004).

5. Ochratoxin A

Ochratoxin A (OTA) is produced by several fungal species, that fall into three distinct groups. *Aspergillus* species related to *Aspergillus ochraceus* (which gives this toxin its name) are xerophiles, and mainly produce OTA in long stored grains. The most common of these species is now believed to be *Aspergillus westerdijkiae*, as *A. ochraceus* has been shown to be only a minor producer. *A. westerdijkiae* was segregated from *A. ochraceus* by Frisvad, Frank, Houbraken, Kuijpers, and Samson (2004), along with *Aspergillus steynii*. The role of the latter species is less well defined. These three species are very closely related, and are often simply regarded as *A. ochraceus*. The second group comprises two species within the black Aspergilli: principally *Aspergillus carbonarius*, with a minor proportion of *A. niger* isolates. *A. niger* is an ubiquitous species in fresh fruits, especially

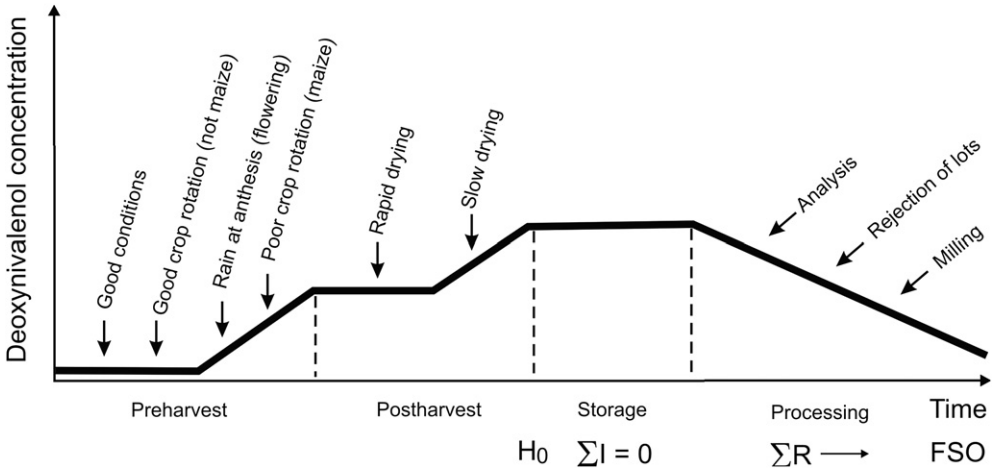

Fig. 8. The time course of deoxynivalenol formation and reduction in wheat, with reference to the Food Safety Objective.

grapes and berries, and some vegetables, including onions, grains, especially maize, and many other food commodities (Pitt & Hocking, 2009). Until the discovery less than 10 years ago that *A. carbonarius* isolates almost all produce OTA, *A. carbonarius* was rarely differentiated from *A. niger*, so its occurrence in foods is less well documented. So far as is known, however, it is much less common in foods than *A. niger*. Some other black *Aspergillus* species also occur in similar habitats, including *Aspergillus tubingensis* (morphologically indistinguishable from *A. niger*), *Aspergillus aculeatus* and *Aspergillus awamori*, but these species do not produce OTA. The third group of OTA producers consists of *Penicillium verrucosum* and the closely related *Penicillium nordicum*. *P. verrucosum* occurs in cool temperate zone grains, while *P. nordicum* is known from cool stored meats. The importance of the latter is unknown and it will not be considered further. These three groups produce toxins under somewhat different conditions, as detailed below.

5.1. Ochratoxin A in coffee

Coffee is grown in elevated tropical regions, as temperatures below 19 °C are required for flowering, but berries mature best near 30 °C. Coffee trees are large perennial shrubs, with cherries developing profusely along the stems. Two main species are cultivated, *Coffea arabica* and *C. robusta*. Some differences have been reported in invasion by *A. ochraceus* (and related species) and *A. carbonarius* (and related species), but the data are limited.

5.1.1. Preharvest

No evidence has been found that *A. carbonarius* or *A. ochraceus* infect immature coffee cherries, so little or no OTA is present in cherries at harvest (Fig. 9).

5.1.2. Postharvest

A consequence of the climate suitable for growing coffee is that drying conditions after harvest are often less than ideal, with mist and rain occurring in many areas. Coffee cherries are traditionally sun dried, and it has been found that these fungal species invade during the drying period (Taniwaki, Pitt, Teixeira, & Iamanaka, 2003; Urbano, Taniwaki, Leitao, & Vicentini, 2001). Differences in geographical area and perhaps coffee species influence which fungal species is dominant. Good drying practice, especially mechanical drying, can essentially eliminate OTA from coffee, however, poor drying conditions will usually result in a positive H_o value (Taniwaki et al., 2003).

5.1.3. Storage

On farm storage, here considered to be before H_o, is a major source of OTA in poorly handled coffee crops (Taniwaki et al., 2003). In general terms, cooperative or warehouse storage is of a higher standard, and little increase in OTA levels normally occurs.

5.1.4. Processing

Processing of coffee cherries is normally confined to sorting of defective (broken and discoloured) cherries by hand or gravity tables. However, modern laser sorters could be used with advantage. Generally, OTA levels in coffee are low, and chemical analysis followed by rejection of poor lots will usually enable meeting the FSO. The roasting process reduces OTA levels in coffee from 8 % to 98 % depending on the time and temperature of roasting (Ferraz et al., 2010).

5.2. Ochratoxin A in dried vine fruits and wines

Despite obvious differences in end products, the formation of OTA in dried vine fruits and wines comes from the same fungal sources, so these products are treated together, with differences emphasised where needed. The source of OTA in these products is *A. carbonarius* (and to a much less extent, *A. niger*). These species grow at high temperatures, up to 41 °C for *A. carbonarius* and 45–47 °C for *A. niger* (Pitt & Hocking, 2009). Grapes grow over a wide climate range, but as the fungal sources grow optimally at higher temperatures, OTA production occurs mostly in warmer areas.

5.2.1. Preharvest

It appears to be unlikely that *A. carbonarius* or *A. niger* are able to infect intact grapes, so under good conditions OTA is not formed in grapes preharvest (Figs. 10 and 11). However, damage to the grape skin permits entry, and these fungi thrive in the high sugar, acid internal environment. Several factors may cause skin damage. First, grapes before harvest may be infected by a range of pathogenic fungi, principally *Botrytis cinerea*, *Rhizopus stolonifer* and powdery mildew (*Erysiphe necator*). Second, some cultivars are prone to skin splitting if rain occurs on the days before harvest. Third, mechanical damage before or at the time of harvest may also permit entry (Leong, Hocking, & Scott, 2006a).

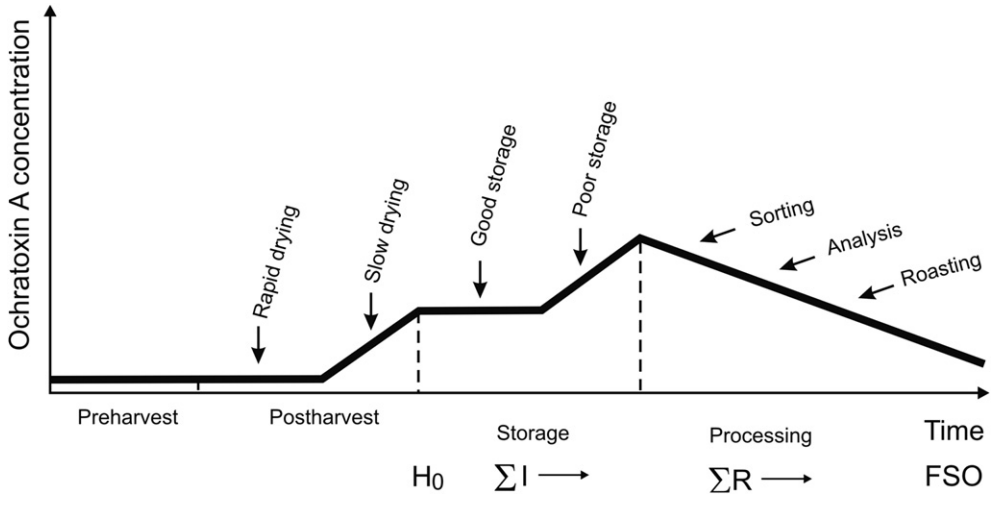

Fig. 9. The time course of ochratoxin A formation and reduction in coffee, with reference to the Food Safety Objective.

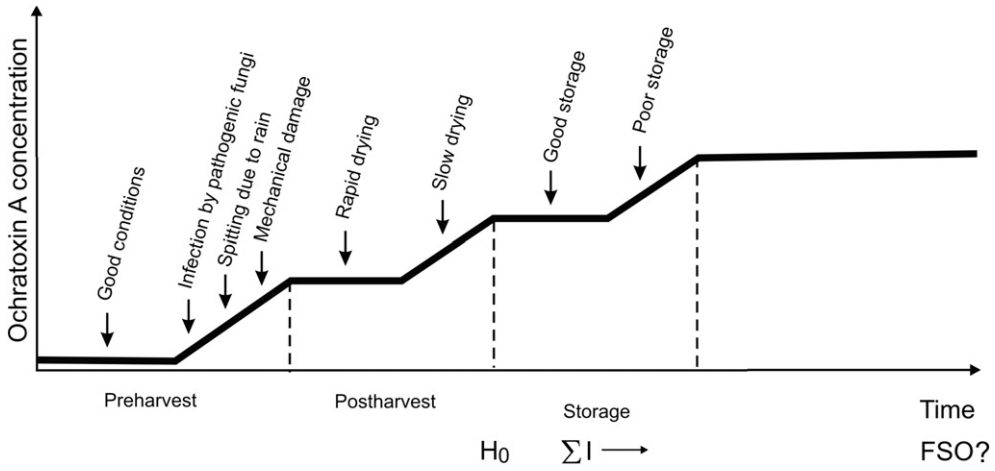

Fig. 10. The time course of ochratoxin A formation in grapes and drying of vine fruits, with reference to the Food Safety Objective.

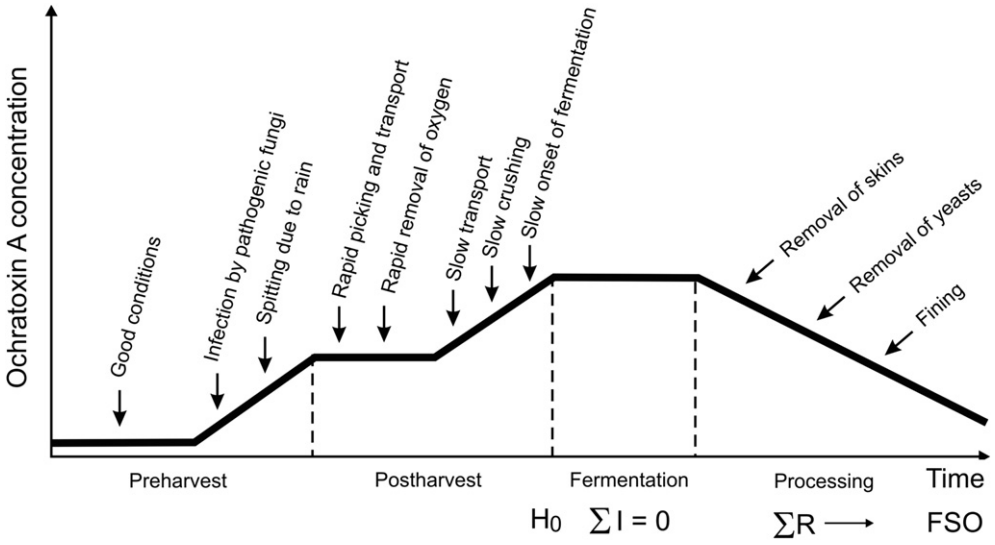

Fig. 11. The time course of ochratoxin A formation in grapes and reduction during wine manufacture, with reference to the Food Safety Objective.

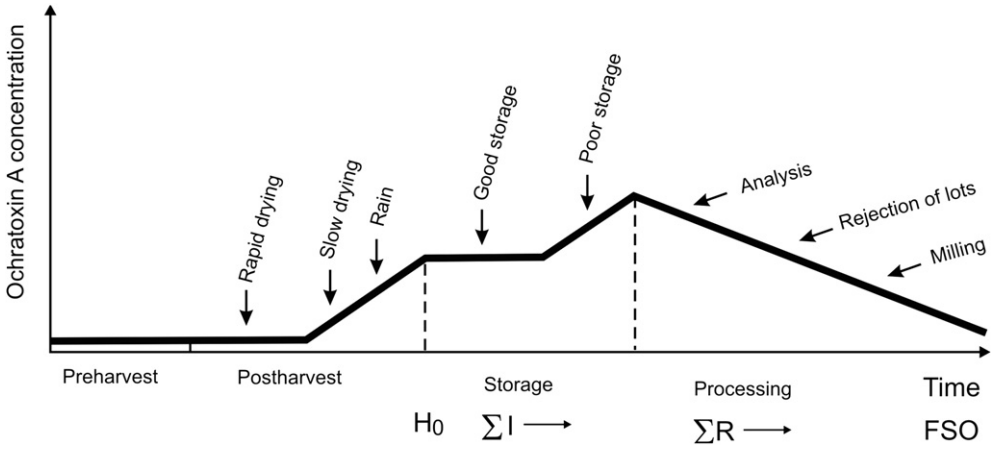

Fig. 12. The time course of ochratoxin A formation and reduction in small grain cereals in Europe, with reference to the Food Safety Objective.

5.2.2. Postharvest

Grapes for drying are usually hand harvested, but mechanical damage may occur at that point or in the various preparation steps used to assist drying. Grapes are normally sun dried on trays or paper, and again mechanical damage is an issue. Black *Aspergillus* species are resistant to sunlight, UV and high temperatures, so OTA is readily produced during grape drying. Both species that produce OTA are moderately xerophilic, so the a_w must be reduced to below 0.8 to prevent OTA formation (Fig. 10).

Grapes for wine production may be mechanically or hand harvested, and are usually transported to a winery at some distance. The fungi continue to grow after crushing, until fermentation produces sufficient carbon dioxide to cause inhibition. This may not occur until one to three days after harvest. Blanketing of the grapes with carbon dioxide before crushing, a common practice in Australia, inhibits growth of these species at an earlier time (Fig. 11).

5.2.3. Storage

Unlike cereals, dried vine fruits contain a high level of sugar, so substantial changes in moisture content are required to cause appreciable changes in a_w. For that reason, adequately dried vine fruits are unlikely to show moisture increases in storage to permit an increase in OTA formation. Normally $\sum I = 0$.

Wines show a slow decrease in OTA level in storage. In practice, $\sum I = 0$.

5.2.4. Processing

Dried vine fruits are normally not processed at all to reduce defects or OTA levels. $\sum R = 0$. The FSO is often attained, as OTA levels in dried vine fruits are usually low – but that is not always the case. The high consumption of dried vine fruits by children as a snack is a cause for concern.

OTA reduction in wine results from removal of skins – so white wines, where skins are removed earlier, usually have lower levels of OTA than red wines. Removal of yeasts and the use of fining agents also cause reduction in OTA levels (Leong, Hocking, & Scott, 2006b, Leong, Hocking, Varelis, Giannikopoulos, & Scott, 2006).

5.3. Ochratoxin A in cereals due to Penicillium verrucosum

The major cause of OTA production in cereals in Europe and Canada is *P. verrucosum*. This species occurs only in cool temperate zones. If OTA is detected in cereals such as maize in Africa, for example, that is due to growth of *A. carbonarius* or *A. niger*, usually a minor problem in comparison with the formation of aflatoxins and fumonisins.

5.3.1. Preharvest

No evidence has been found to support the theory that *P. verrucosum* produces OTA in European or Canadian cereals as the result of preharvest invasion. Infection appears to be strictly postharvest, during the drying stage (Olsen et al., 2004, 2006, Tittlemeir, Roscoe, Blagden, Kobialka, & Nowicki, 2012).

5.3.2. Postharvest

Slow drying under less than ideal conditions of temperature, often in conjunction with rain or fog, appears to be the main factor permitting the growth of *P. verrucosum* in European or Canadian barley and wheat (Olsen et al., 2004, 2006). Increases in $\sum I$ are common (Fig. 12).

5.3.3. Storage

It is not clear whether OTA can increase in storage of small grains in Europe. *P. verrucosum* is a xerophile, so such a possibility cannot be discounted.

5.3.4. Processing

Analysis for OTA and rejection of substandard lots for animal feed appear to be the only way to reduce OTA in European of Canadian small grains. Inspection ensures that the FSO is met for human foods.

References

Anderson, N. M., Larkin, J. W., Cole, M. B., Skinner, G. E., Whiting, R. C., Gorris, L. G. M., et al. (2011). Food safety objective approach for controlling *Clostridium botulinum* growth and toxin production in commercially sterile foods. *Journal of Food Protection, 74*, 1956–1989.

Bakan, B., Melcion, D., Richard-Molard, D., & Cahagnier, B. (2002). Fungal growth and *Fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. *Journal of Agricultural and Food Chemistry, 50*, 728–731.

Baker, S. E. (2006). *Aspergillus niger* genomics: past, present and into the future. *Medical Mycology, 44*, S17–S21.

Bansal, J., Pantozopoulos, P., Tam, J., Cavlovic, P., Kwong, K., Turcotte, A. M., et al. (2011). Surveys of rice sold in Canada for aflatoxins, ochratoxin A and fumonisins. *Food Additives and Contaminants A, 28*, 767–774.

Borompichaichartkul, C., Srzednicki, G. S., & Driscoll, R. H. (2003). Drying at subzero temperatures: case study on in-store drying of grains. *Drying Technology, 21*, 735–754.

Bullerman, L. B., & Bianchini, A. (2007). Stability of mycotoxins during food processing. *International Journal of Food Microbiology, 119*, 140–146.

Bullerman, L. B., Bianchini, A., Hanna, M. A., Jackson, L. S., Jablonski, J., & Ryu, D. (2008). Reduction of fumonisin B_1 in corn grits by single-screw extrusion. *Journal of Agricultural and Food Chemistry, 56*, 2400–2405.

CAC (Codex Alimentarius Commission). (2007). *Principles and guidelines for the conduct of microbiological risk management and its annex on guidance on microbiological risk management metrics*. CAC/GL 63-2007. http://www.ilri.org/safefood/files/Codex%20principles%20microbiological%20RM.pdf Accessed 12.04.12.

Calderari, T. O., Iamanaka, B. T., Frisvad, J. C., Pitt, J. I., Sartori, D., Pereira, J. L., et al. The biodiversity of Aspergillus section Flavi in Brazil nuts: from rainforest to consumer. *Journal of Food Microbiology*, in press.

Campbell, B. C., Molyneux, R. J., & Schatzki, T. F. (2003). Current research on reducing pre- and post-harvest aflatoxin contamination on US almond, pistachio and walnut. *Journal of Toxicolology Toxin Reviews, 22*, 225–266.

Chauhan, Y. S., Wright, G. C., Rachaputi, R. C. N., Holzworth, D., Broome, A., Krosch, S., et al. (2010). Application of a model to assess aflatoxin risk in peanuts. *Journal of Agricultural Science, 148*, 341–351.

De la Campa, R., Miller, J. D., & Hendricks, K. (2004). Fumonisin in tortillas produced in small-scale facilities and effect of traditional masa production methods on this mycotoxin. *Journal of Agricultural and Food Chemistry, 52*, 4432–4437.

Delwiche, S. R., Pearson, T. C., & Brabec, D. J. (2005). High-speed optical sorting of soft wheat for reduction of deoxynivalenol. *Plant Disease, 89*, 1214–1219.

Doko, M. B., Rapior, S., Visconti, A., & Schjoth, J. E. (1995). Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. *Journal of Agricultural and Food Chemistry, 43*, 429–434.

Dorner, J. W., Cole, R. J., & Blankenship, P. D. (1992). Use of a biocompetitive agent to control preharvest aflatoxin in drought stressed peanuts. *Journal of Food Protection, 55*, 888–892.

Dorner, J. W., Cole, R. J., & Blankenship, P. D. (1998). Effect of inoculum rate of biological control agents on preharvest aflatoxin contamination of peanuts. *Biological Control, 12*, 171–176.

Doster, M. A., & Michailides, T. J. (1994). *Aspergillus* molds and aflatoxins in pistachio nuts in California. *Phytopathology, 84*, 583–590.

Doster, M. A., & Michailides, T. J. (1995). The relationship between date of hull splitting and decay of pistachio nuts by *Aspergillus* species. *Plant Disease, 79*, 766–769.

Dowd, P. F. (2001). Biotic and abiotic factors limiting efficacy of Bt corn in indirectly reducing mycotoxin levels in commercial fields. *Journal of Economic Entomology, 94*, 1067–1074.

Ferraz, M. B. M., Farah, A., Iamanaka, B. T., Perrone, D., Copetti, M. V., Marques, V. X., et al. (2010). Kinetics of ochratoxin A destruction during coffee roasting. *Food Control, 21*, 872–877.

Frisvad, J. C., Frank, J. M., Houbraken, J. A. M. P., Kuijpers, A. F. A., & Samson, R. A. (2004). New ochratoxin A producing species of *Aspergillus* section *Circumdati*. *Studies in Mycology, 50*, 23–43.

Frisvad, J. C., Smedsgaard, J., Samson, R. A., Larsen, T. O., & Thrane, U. (2007). Fumonisin B_2 production by *Aspergillus niger*. *Journal of Agricultural and Food Chemistry, 55*, 9727–9732.

García-Cela, E., Ramos, A. J., Sanchis, V., & Marin, S. (2012). Emerging risk management metrics in food safety: FSO, PO. How do they apply to the mycotoxin hazard? *Food Control, 25*, 797–808.

Hazel, C. M., & Patel, S. (2004). Influence of processing on trichothecene levels. *Toxicology Letters, 153*(S1), S51–S59.

ICMSF (International Commission on Microbiological Specifications for Foods). (2002a). *Microorganisms in foods. 7. Microbiological testing in food safety management*. New York: Kluwer Academic/Plenum Press.

ICMSF (International Commission on Microbiological Specifications for Foods). (2002b). Aflatoxins in peanuts. In *Microorganisms in foods. 7. Microbiological testing in food safety management* (pp. 263–271). New York: Kluwer Academic/Plenum Press.

Jackson, L. S., Jablonski, J., Bullerman, L. B., Bianchini, A., Hanna, M. A., Voss, K. A., et al. (2011). Reduction of fumonisin B_1 in corn grits by twin-screw extrusion. *Journal of Food Science, 76*, T150–T155.

Khlangwiset, P., Shephard, G. S., & Wu, F. (2011). Aflatoxins and growth impairment: a review. *Critical Reviews in Toxicology, 41*, 740–755.

Leong, S.-L., Hocking, A. D., & Scott, E. S. (2006a). Survival and growth of *Aspergillus carbonarius* on wine grapes before harvest. *Journal of Food Microbiology, 111*, S83–S87.

Leong, S.-L., Hocking, A. D., & Scott, E. S. (2006b). The effect of juice clarification, static or rotary fermentation and fining on ochratoxin A in wine. *Australian Journal of Grape and Wine Research, 12*, 245–251.

Leong, S.-L. L., Hocking, A. D., Varelis, P., Giannikopoulos, G., & Scott, E. S. (2006). Fate of ochratoxin A during vinification of semillon and shiraz grapes. *Agricultural and Food Chemistry, 54*, 6460–6464.

Marklinder, I., Lindblad, M., Gidlund, A., & Olsen, M. (2005). Consumers' ability to dicriminate aflatoxin-contaminated Brazil nuts. *Food Additives and Contaminants, 22*, 56–64.

Michailides, T. J. (1989). The "Achilles Heel" of pistachio fruit. *California Agriculture, 43*, 10–11.

Miller, J. D. (1994). Epidemiology of Fusarium ear diseases of cereals. In J. D. Miller, & H. L. Trenholm (Eds.), *Mycotoxins in grain – Compounds other than aflatoxin* (pp. 19–36). St. Paul, MN: Eagan Press.

Miller, J. D. (1995). Fungi and mycotoxins in grains, implications for stored products research. *Journal of Stored Products Research, 31*, 1–16.

Munkvold, G. P., Hellmich, R. L., & Rice, L. P. (1999). Comparison of fumonisin concentrations in kernels of transgenic BT maize hybrids and nontransgenic hybrids. *Plant Disease, 83*, 130–138.

Munkvold, G. P., Hellmich, R. L., & Showers, W. B. (1997). Reduced Fusarium ear rot and symptomless infections of maize genetically engineered for European corn borer resistance. *Phytopathology, 87*, 1071–1077.

Njapau, H., Muzungaile, E. M., & Changa, R. C. (1998). The effects of village processing techniques on the content of aflatoxins in corn and peanuts in Zambia. *Journal of the Science of Food and Agriculture, 76*, 450–456.

Olsen, M., Johnsson, P., Moller, T., Paladino, R., & Lindblad, M. (2008). *Aspergillus nomius*, an important aflatoxin producer in Brazil nuts? *World Mycotoxin Journal, 1*, 123–126.

Olsen, M., Jonsson, N., Magen, N., Banks, J., Fanelli, C., Rizzo, A., Haikara, A., Dobson, A., Frisvad, J., Holmes, S., Olkku, J., Persson, S.-J., & Börjesson, T. (2004). *Prevention of ochratoxin A in cereals (OTAPREV)*. EU report QLK1-CT-1999-00433.

Olsen, M., Jonsson, N., Magen, N., Banks, J., Fanelli, C., Rizzo, A., et al. (2006). Prevention of ochratoxin A in cereals in Europe. In A. D. Hocking, J. I. Pitt, R. A. Samson, & U. Thrane (Eds.), *Advances in food mycology* (pp. 317–348). New York: Springer.

Pearson, T. C., Doster, M. A., & Michailides, T. J. (2001). Automated detection of pistachio defects by machine vision. *Applied Engineering in Agriculture, 17*, 729–732.

Pietri, A., Zanetti, M., & Bertuzzi, T. (2009). Distribution of aflatoxins and fumonisins in dry-milled maize fractions. *Food Additives and Contaminants, 26*, 372–380.

Pitt, J. I. (1989). Field studies on *Aspergillus flavus* and aflatoxins in Australian groundnuts. In *Aflatoxins in groundnut: proceedings of the International Workshop, 6–9 October, l987, ICRISAT Center, India* (pp. 223–235). Patancheru, India: ICRISAT.

Pitt, J. I. (2004). Biocontrol of aflatoxins in peanuts. In D. Barug, H. van Egmond, R. Lopez-Garcia, T. van Osenbruggen, & A. Visconti (Eds.), *Meeting the mycotoxin menace* (pp. 141–152). Wageningen, Netherlands: Wageningen Academic Publishers.

Pitt, J. I., & Hocking, A. D. (1996). Current knowledge of fungi and mycotoxins associated with food commodities in Southeast Asia. In E. Highley, & G. I. Johnson (Eds.), *Mycotoxin contamination in grains* (pp. 5–10). Canberra: Australian Centre for International Agricultural Research.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (3rd ed.). New York: Springer.

Pitt, J. I., Hocking, A. D., Bhudhasamai, K., Miscamble, B. F., Wheeler, K. A., & Tanboon-Ek, P. (1994). The normal mycoflora of commodities from Thailand. 2. Beans, rice, small grains and other commodities. *International Journal of Food Microbiology, 23*, 35–53.

Rios, G., Pinson-Gadais, L., Abecassis, J., Zakhia-Rozis, N., & Lullien-Pellerin, V. (2009). Assessment of dehulling efficiency to reduce deoxynivalenol and *Fusarium* level in durum wheat grains. *Journal of Cereal Science, 49*, 387–392.

Schaafsma, A. W., & Hooker, D. C. (2007). Climatic models to predict occurrence of *Fusarium* toxins in wheat and maize. *International Journal of Food Microbiology, 119*, 116–125.

Schaafsma, A. W., Tamburic-Ilincic, L., & Hooker, D. C. (2005). Effect of previous crop, tillage, field size, adjacent crop, and sampling direction on airborne propagules of *Gibberella zeae/Fusarium graminearum*, Fusarium head blight severity, and deoxynivalenol accumulation in winter wheat. *Canadian Journal of Plant Pathology, 27*, 217–224.

Schatzki, T. F., & Ong, M. S. (2000). Distribution of aflatoxin in almonds. 2. Distribution in almonds with heavy insect damage. *Journal of Agriculture and Food Chemistry, 48*, 489–492.

Schatzki, T. F., & Ong, M. S. (2001). Dependence of aflatoxin in almonds on the type and amount of insect damage. *Journal of Agriculture and Food Chemistry, 49*, 4513–4519.

Schatzki, T. F., & Pan, J. L. (1996). Distribution of aflatoxin in pistachios. 3. Distribution in pistachio process streams. *Journal of Agricultural and Food Chemistry, 44*, 1076–1084.

Siriacha, P. (1991). *Control of aflatoxin contamination of maize in Thailand*. PhD Thesis. Tokyo: Tokyo University of Agriculture.

Sommer, N. F., Buchanan, J. R., & Fortlage, R. J. (1986). Relation of early splitting and tattering of pistachio nuts to aflatoxin in the orchard. *Phytopathology, 76*, 692–694.

Takahashi, H., Okana, S., & Ichinoe, M. (2001). Present situation for production of pistachio nuts in Iran. *Mycotoxins, 51*, 95–102.

Taniwaki, M. H., Pitt, J. I., Teixeira, A. A., & Iamanaka, B. T. (2003). The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. *Journal of Food Microbiology, 82*, 173–179.

Thammawong, M., Okabe, M., Kawasaki, T., Nakagawa, H., Nagashima, H., Okadome, H., et al. (2010). Distribution of deoxynivalenol and nivalenol in milling fractions from *Fusarium*-infected Japanese wheat cultivars. *Journal of Food Protection, 73*, 1817–1823.

Tittlemeir, S. A., Roscoe, M., Blagden, R., Kobialka, C., & Nowicki, T. (2012). An overview of ochratoxin A in Canadian grains. *Canadian Journal of Plant Pathology, 34*, 346.

Urbano, G. R., Taniwaki, M. H., Leitao, M. F. D., & Vicentini, M. C. (2001). Occurrence of ochratoxin A-producing fungi in raw Brazilian coffee. *Journal of Food Protection, 64*, 1226–1230.

Visconti, A. (1996). Fumonisins in maize genotypes grown in various geographic areas. *Advances in Experimental Medicine and Biology, 392*, 193–204.

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: a review of toxicology. *American Journal of Clinical Nutrition, 80*, 1106–1122.

Yazdanpanah, H., Mohammadi, T., Abouhossain, G., & Cheraghali, A. M. (2005). Effect of roasting on degradation of aflatoxins in contaminated pistachio nuts. *Food and Chemical Toxicology, 43*, 1135–1139.

Zwietering, M. H., Stewart, C. M., Whiting, R. C., & International Commission on Microbiological Specifications for Foods. (2010). Validation of control measures in a food chain using the FSO concept. *Food Control, 30*, 1–7.