CHAPTER 5

Chemical Hazards and Their Control: Toxins

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MICROBIAL TOXINS

Many food commodities are susceptible to contamination by microorganisms, the subsequent growth of which can lead to the presence of toxic compounds in food. Some of the bacterial toxins, such as those of *Staphylococcus aureus*, *Clostridium botulinum*, and many Gram-negative species associated with food poisoning are macromolecules such as proteins. However, some bacteria are able to produce low molecular weight toxic metabolites, which may contaminate foods and cause serious poisoning. One example is *Burkholderia* (*Pseudomonas*) *cocovenenans*, which produces toxins such as bonkrekic acid and toxoflavin (see bongkrek poisoning on page 112). Another example is the macrocyclic depsipeptide, cereulide, which is recognized as the emetic toxin of *Bacillus cereus*.

Several species of cyanobacteria can also produce very toxic metabolites, but rarely are these directly associated with foods. An exception may be the contamination of species, such as *Spirulina*, grown as food or as components of "health food" products, with toxigenic species. More directly associated with foods are the toxins that are produced by groups of eukaryotic algae such as dinoflagellates and diatoms. These may be ingested by shellfish or fish and pass through the food chain to humans.

A number of species of fungi can produce relatively low molecular weight secondary metabolites, which are toxic to humans and domesticated animals and are referred to as mycotoxins.[49] Mycotoxin biosynthesis may be associated with the preharvest stage of crop production by fungi that are obligate endophytes of plants, plant pathogens, or members of the flora responsible for the decay of senescent plant material.[47] However, the highest concentrations of many of these toxic metabolites are produced by fungi growing on postharvest commodities that are stored under inappropriate conditions.[31] The majority of mycotoxins are especially important in the context of animal husbandry, but several are also significant as contaminants of human foods and will be dealt with in the following sections. Table 5–1 summarizes the toxins considered, their most common sources, and the food commodities most frequently implicated.

A number of fermented foods involve a mold-ripening stage, usually with species of *Penicillium*. Some of these molds are known to be potentially toxigenic; thus, strains of *P. roqueforti* can produce PR-toxin but not during the production of the blue cheeses. Many strains of *P. camemberti* produce cyclopiazonic acid, and this compound has been detected in the crusts of Camembert-type cheese but not in the interior of the cheeses.[35,36] Cyclopiazonic acid has also been found associated with mold-ripened fermented sausage.[76] *Aspergillus versicolor* is a common member of the surface flora of hard cheeses stored for long periods, and this species is known to produce sterigmatocystin, a mycotoxin that is a biosynthetic precursor of the aflatoxins and is both acutely toxic and carcinogenic but very much less so than aflatoxin B_1.

Table 5–1 Toxic Microbial Metabolites that May Be Associated with Foods

Toxin	*Sources*	*Commodities*	*LD_{50} (mg/kg)*
Aflatoxin B_1	*Aspergillus flavus* *A. parasiticus* *A. nomius*	Maize, groundnuts, treenuts, dried figs, spices	0.5 (dog) 9.0 (mouse)
Ochratoxin A	*Penicillium verrucosum* *A. ochraceus*	Cereals, coffee, spices, dried vine fruits	28 (rat)
Patulin	*P. expansum* *A. clavatus*	Apple juice, other fruit juices, malted barley residues	35 (mouse)
T-2 toxin	*Fusarium sporotrichioides*	Overwintered cereals	5.2 (rat)
Deoxynivalenol	*F. graminearum*	Cereals	46 (mouse)
Fumonisin B_1	*F. moniliforme*	Maize	?
Cyanoginosin	*Microcystis aeruginosa*	Water	0.05 (mouse)
Bonkrekic acid	*Burkholderia cocovenenans*	Tempeh bonkrek	6.84 (oral LD_{100} in mouse)
Toxoflavin	*B. cocovenenans*	Tempeh bonkrek	8.4 (oral in mouse)
Saxitoxin	*Alexandrium catenella*	Mussels	0.012 (ip in mouse)
Okadaic acid	*Dinophysis fortii*	Mussels	0.2 (LD_{99} in mouse)
Domoic acid	*Pseudonitzschia pungens*	Mussels	3.6 (mouse)

This mycotoxin can be found in cheeses but usually only at the surface.[52] A recent study has demonstrated the occurrence of sterigmatocystin in Ras cheese that was purchased in local markets in Egypt.[43]

AFLATOXINS

Although aflatoxins are produced by a small number of species of the genus *Aspergillus*, they are especially widespread because there are essentially three routes to the contamination of food commodities.

1. Direct contamination through the mold spoilage of stored products by species such as *A. flavus*, *A. parasiticus,* and *A. nomius*
2. Preharvest production in the field by the establishment of an endophytic association of one of these species with plants such as maize and groundnuts, followed by some form of stress on the growing crop, such as drought
3. Passage through the food chain into animal products, such as milk, following the consumption by farm animals of contaminated animal feeds

The aflatoxins are a family of metabolites; the most important of which in terms of both toxicity and prevalence is aflatoxin B_1 (Figure 5–1). Aflatoxins are acutely toxic, carcinogenic, and immunosuppressive. Aflatoxins have been found in a wide range of tropical and subtropical products such as figs, pistachio and Brazil nuts, spices, peanuts, and maize. Pittet[59] provided a useful update on the natural occurrence of aflatoxins and other mycotoxins with detailed information concerning the incidence and range of concentrations found. The most important of those commodities that might be used as a raw material for fermented foods is maize, although there have been reports of low concentrations of aflatoxins in both rice[50] and wheat.[56] A diverse range of products associated with foods and beverages of the Far East, such as rice wine, soy sauce, and miso, involves the use of a source of amylolytic, lipolytic, and proteolytic enzymes. This material, known as *koji*, traditionally is produced by growing appropriate strains of *A. oryzae* on substrates such as rice, wheat, and soya beans. This mold is closely related to *A. flavus*, and may indeed be a "domesticated" form of this species.[15] Some koji fungi are indistinguishable from *A. flavus* but are nontoxigenic. It seems that the selection of strains producing increased levels of secreted hydrolytic enzymes has selected strains that do not produce aflatoxins.

Aflatoxins are susceptible to both microbial and mammalian metabolism. Indeed, it is a consequence of metabolism in the mammalian liver that the aflatoxins are toxic (Figure 5–2). The wide range in both acute and carcinogenic toxicity (Table 5–2) in different animal species results from differences in the metabolic activities of these animals. Carcinogenicity is associated with the formation of aflatoxin epoxide and its subsequent reaction with guanine residues in DNA, whereas the acute toxicity requires the hydroxylation of this epoxide to form dihydroxyaflatoxin, which can react with the lysine residues of proteins. The possibility that microorganisms could be used to degrade aflatoxins, and hence detoxify contaminated foods, has been reviewed.[5] One of the earliest reports of the successful removal of aflatoxins by microorganisms was that of Lillehoj and his colleagues at the Northern Regional Research Laboratories in Peoria, Illinois.[37,38] Arising from a wide screen of both eukaryotic and prokaryotic microorganisms, the bacterium *Flavobacterium aurantiacum* (NRRL B-184) was the most effective, but the phenomenon has not been converted into a practical detoxification process.

When cows are fed on feed contaminated with aflatoxin B_1, they secrete a proportion of the contaminant in their milk as the metabolite, aflatoxin M_1. This compound is less toxic than its precursor, but, because it is uniformly distributed in a liquid food such as milk, and very young and elderly people may be exposed to a significant extent, the European Commission (EC) has set a particularly stringent maximum permissible level for aflatoxin M_1 in milk and dairy products of 0.05 μg/kg. The comparable level for aflatoxin B_1 in groundnuts, nuts, dried fruit, and cereals for direct human consumption is 2 μg/kg (EC No 1525/98), although less stringent levels are set in other parts of the world.[8] Unfortunately, there is no consistent evidence that aflatoxin M_1 is removed during any of the most commonly used processes in the milk industry, such as heat treatment, cold storage, or spray drying.[79] Neither is aflatoxin completely degraded during the fermentation processes used in the manufacture of cheese, cream, or butter, although it may be partitioned between the different components of each process. Thus,

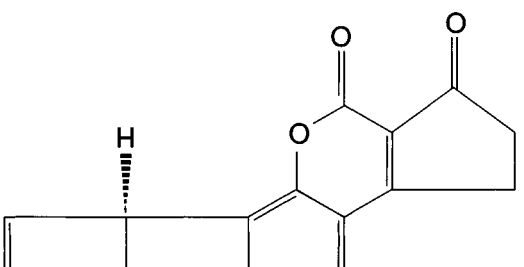

Figure 5–1 Aflatoxin B_1.

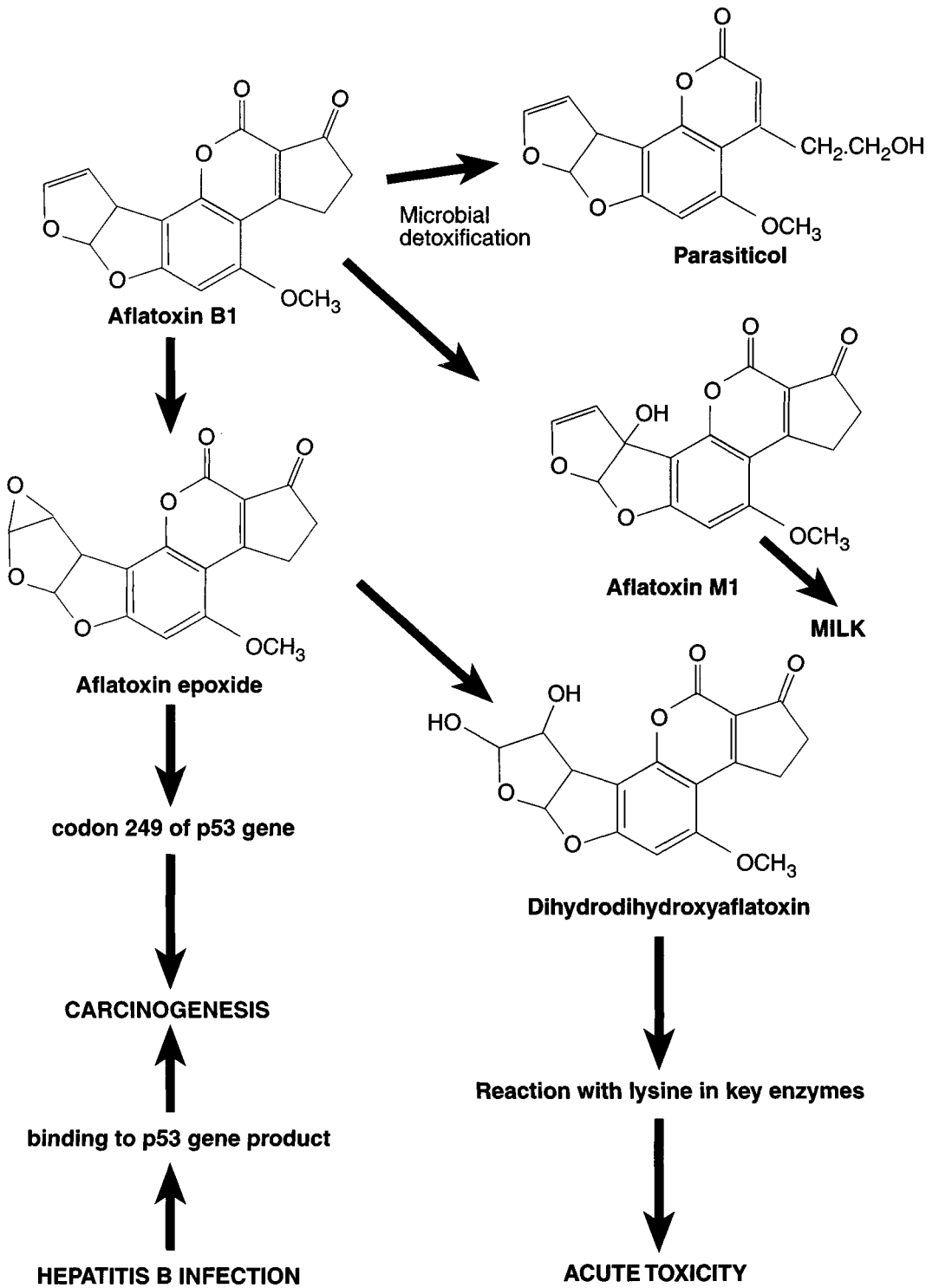

Figure 5–2 The relationship between metabolism and toxicology of aflatoxin B_1.

Table 5–2 Oral Acute LD_{50} Values and TD_{50} Values for Carcinogenesis of Aflatoxin B_1

Animal Species	*LD_{50} (mg/kg body wt)*	*TD_{50} (μg/kg body wt/day)*
Rabbit	0.3	–
Cat	0.6	–
Dog	0.5–1.0	–
Pig	0.6	–
Baboon	2.0	–
Rat (male)	5.5	1.3–5.8
Rat (female)	17.9	6.9–12.5
Macaque monkey	7.8	–
Rhesus monkey		156
Cynamolgus monkey		848
Mouse	9.0	>5,300
Hamster	10.2	–
Humans	5.0*	132†

*Based on epidemiological evidence from cases of acute poisoning[31]
†Based on an analysis of intake versus incidence data[78]

cheese may retain as much as 60% of the aflatoxin M_1 in the milk that is used in its manufacture, whereas cream may retain only approximately 10%, and butter as little as 2%.

Aflatoxin B_1 may be detoxified during the fermentation of milk because *Lactococcus lactis* (previously known as *Streptococcus lactis*) is able to convert it into aflatoxin B_{2a} and aflatoxicol (Figure 5–3).[40,41] In fact, the formation of aflatoxin B_{2a} may simply be the result of the reduced pH; there is the possibility of it being reconverted to the parent compound. However, it has been claimed that the formation of aflatoxin B_{2a} is not simply a result of acid-catalyzed hydration of aflatoxin B_1, and also that viability is not a prerequisite for the removal of aflatoxin B_1 by probiotic strains of *Lactobacillus rhamnosus*.[20,21] There have been searches for other microbial systems for the removal of aflatoxins from foods and a possibility actively being investigated is the use of enzymes from a nontoxic, edible species of fungus, *Armillaria tabescens*, which is valued already in China for its medicinal properties in alleviating a number of disorders.[39]

An alternative strategy to detoxifying aflatoxin directly in foods is the possibility of removal from the gastrointestinal tract by probiotic bacteria. Such a study has been carried out using probiotic strains of *Lactobacillus* and *Propionibacterium*.[2] The results from these investigations suggest that such probiotic bacteria have a role in reducing the bioavailability of foodborne carcinogens such as aflatoxin B_1. However, the most effective strategies for limiting human exposure to aflatoxins are to avoid contamination in the first place or a chemical process, such as ammoniation, to degrade aflatoxin irreversibly in animal feeds.[63]

OCHRATOXIN A

Ochratoxin A (Figure 5–4) is produced by *P. verrucosum* in temperate climates and by a number of *Aspergillus* species, especially *A. ochraceus*, in warmer parts of the world.[48] Ochratoxin A is most common in cereals of temperate countries, such as barley, oats, rye, and wheat,[30,45] but has also been found in maize,[32] coffee, cocoa, dried vine fruits, wine,[59] and beer.[65] Although ochratoxin A can survive fermentation processes, it normally does not survive the malting process used in the production of beer.[33] Its presence in beer at very low concentrations (ca 0.2 ng/ml) may be due to its pres-

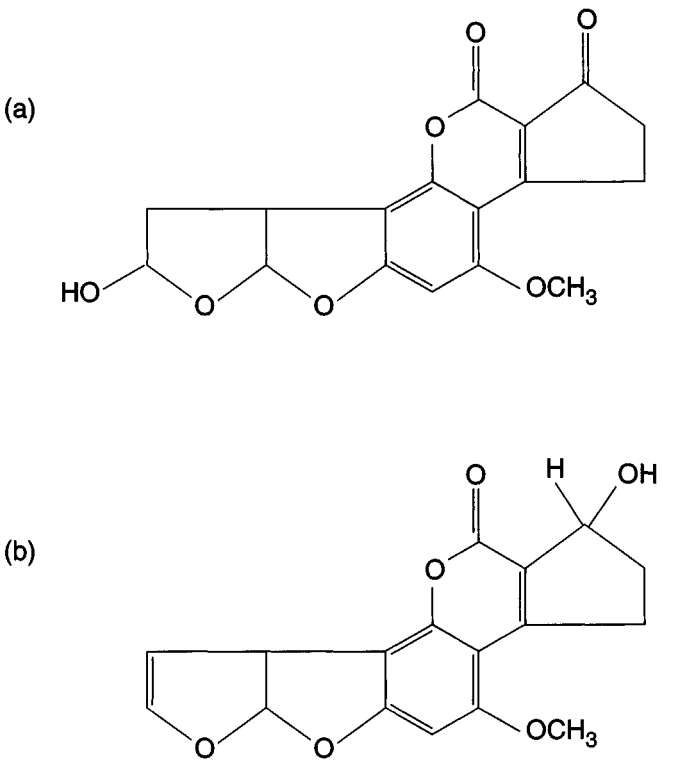

Figure 5–3 (a) Aflatoxin B_{2a} (b) Aflatoxicol.

ence in adjuncts that are used in commercial beer production. A survey of the occurrence of ochratoxins in a range of wines from the Swiss retail market showed them to be present at very low concentrations and to be more frequent, and at higher concentrations, in red wines from more southerly regions of Europe.[82] It is probable that contamination precedes the fermentation stage. These results confirm that ochratoxins are not removed by an alcoholic fermentation.

Ochratoxin A is relatively thermostable, having a half life at 100 °C of more than 10 hours in

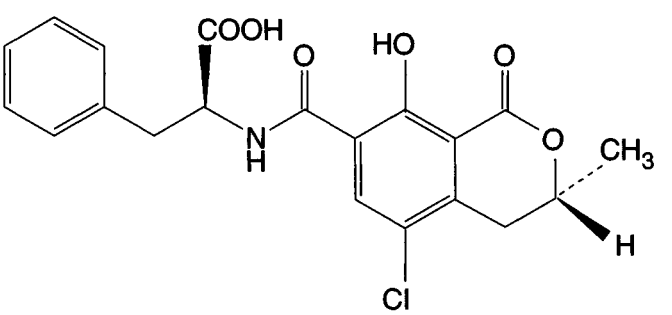

Figure 5–4 Ochratoxin A.

dry wheat and nearly 2.5 hours in moist wheat.[7] Several studies have confirmed that ochratoxin A will survive in most food processes involving a heating stage. Like aflatoxin, ochratoxin A can also pass through the food chain and may be found in meat products, especially of the pig,[32] but it does not seem to be secreted effectively into cow's milk.[75] However, surveys in Scandinavian countries have shown the occurrence of ochratoxin A in cow's milk in Sweden[9] and Norway.[72] Because of the low biotransfer of ochratoxin A from animal feeds to milk, Skaug[72] speculated that the inhalation of contaminated airborne particles may be the route into cow's milk. If ochratoxin is not normally present in milk, it would not be expected to occur in dairy products. However, some cheeses are readily contaminated with molds, and some of the molds isolated from cheeses have been shown to produce ochratoxin in the laboratory. Scott,[64] in his detailed review of the occurrence of mycotoxins in dairy products, concluded that "cheese is generally a good substrate for fungal growth but a poor substrate for experimental mycotoxin production." (p221) There are a few reports of the natural occurrence of ochratoxin A in moldy cheese, and these are referenced in this review, but the molds used deliberately for the production of mold-ripened cheeses (i.e., *P. roqueforti* and *P. camemberti*) do not produce this mycotoxin.

The transfer of ochratoxin from animal feeds to animal tissues, such as muscle, liver, and kidneys, combined with the extended residence time for ochratoxin A in animal tissues, leads to the possibility of its presence in meat products. There are many reports of the occurrence of ochratoxin A in kidneys, liver, and even sausages, and these have been documented extensively.[34] The contamination of meat products by transfer from animal feeds should be distinguished from the occurrence of ochratoxin A in moldy meat products such as smoked pork, other smoked meats, and sausages, in which much higher levels of ochratoxin A can be found.[34]

At the acute level, ochratoxin A is a nephrotoxin, which is certainly responsible for most cases of porcine nephropathy and has been suspected to be an etiological agent in Balkan endemic nephropathy. A detailed risk assessment of this mycotoxin has been carried out[34] and it seems prudent to assume that it is also carcinogenic.[17] For this reason, and because there is no doubt regarding human exposure to ochratoxin A from a range of foods, the member countries of the European Union are presently seeking to agree to maximum levels in foods for human consumption; these are likely to include maximum acceptable levels in fermented beverages such as beer and wine.

PATULIN

Patulin (Figure 5–5) is produced by a number of species of *Penicillium*, *Aspergillus,* and *Byssochlamys,* but, in the context of human foods, the most important species is *P. expansum*. This mold is associated especially with a soft rot of apples, but may also occur on a wide range of other fruits. *P. expansum* is able to form a rapid brown soft rot in apples, once infection has been established, and it becomes immediately recognizable once the conidiophores bearing large numbers of blue green spores form as pustules on the surface of the rot. Although infection is usually through a wound and the rot is usually very evident, some apple varieties can be infected from within, and, although the apple looks superficially sound, the core may be infected and contaminated with patulin. It is unlikely that fresh fruit will be a hazard because as soon as mold contamination is apparent, it will normally be discarded because of the obvious visual and taste defects. However, an organolep-

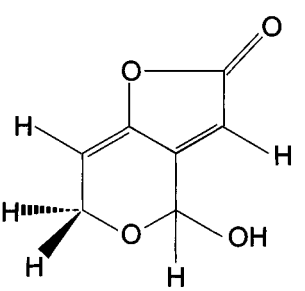

Figure 5–5 Patulin.

tically acceptable fruit juice can be expressed from fruit containing some rot, and patulin is stable at the low pH values of most fruit juices.

The natural occurrence of patulin in commercial apple juice was reported as long ago as 1972.[69] Surveys since then have demonstrated that the contamination of fruit juices with patulin is a continuing nuisance.[10,44,61] Patulin was discovered originally as a potentially useful broad spectrum antibiotic, but the acute toxicity precluded its use. The report of Dickens & Jones in 1961[19] suggested that, at high enough doses, patulin could induce sarcomas in experimental animals at the site of injection and hence may be carcinogenic. However, several studies since then have failed to provide conclusive evidence that patulin is carcinogenic,[26] but the United Kingdom has set an advisory maximum level of 50 μg/l in apple juice for human consumption, and there is not yet any statutory limit for patulin in the European Community. A report of the U.K. Ministry of Agriculture, Fisheries and Food[44] demonstrated that a few samples of fresh apple juice taken from retail outlets in the United Kingdom were contaminated with patulin at levels that were above the advisory limit. A detailed assessment of this report and others, as well as the analytical difficulties associated with the determination of low concentrations of patulin in apple juice, is available.[71]

It has been known for some time that patulin disappears during the fermentation of apple juice to cider using the yeast *Saccharomyces cerevisiae*.[23] The microbial decomposition of patulin requires the yeast to be viable; it is an inducible phenomenon, and occurs during fermentative metabolism rather than respiratory metabolism. More recently, it has been shown that patulin is metabolized to a number of products including ascladiol during a yeast fermentation.[71] If due diligence is paid to the quality of apple juice used in the manufacture of cider, it seems unlikely that patulin will be a problem in cider.

FUSARIUM TOXINS

The genus *Fusarium* contains several species that are important plant pathogens causing serious losses in agriculture and horticulture. They are thus of special importance in the field during the growth and development of a crop. Some species are also capable of continuing growth and metabolic activity postharvest, but they require higher water activities (A_w) than most species of *Penicillium* and *Aspergillus*. *Fusarium* is associated with a very wide range of secondary metabolites, many of which are toxic to farm animals and humans. The three groups that are particularly important in human foods are the polyketide-derived zearalenone, the sesquiterpenoid trichothecenes, and the recently discovered fumonisins. Bennett & Richard[4] reviewed the effects of processing on these toxins in contaminated grains and it is clear that they are stable during wet and dry milling and ethanol fermentations with the exception of deoxynivalenol, for which there is conflicting evidence concerning the effect of fermentation. Although these processes may not destroy the common fusarial toxins, they do influence their segregation among the various fractions of the process. Thus, for example, wet milling will produce toxin-free starch from maize, but the other products, used in animal feeds, will have higher levels of zearalenone than the starting material.

Zearalenone

Zearalenone (Figure 5–6) has little or no acute toxicity but has potent estrogenic activity and is responsible for a serious disorder in pigs known as vulvovaginitis. Zearalenone is produced by several species of *Fusarium*, but the most important are *F. graminearum* and *F. culmorum*, both of which may be responsible for head rot (often characterized by a pink or red discoloration of the de-

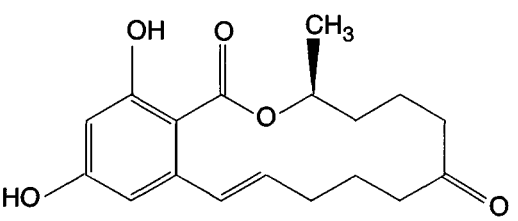

Figure 5–6 Zearalenone.

veloping grain) in cereals such as maize, wheat, and barley. The ecology of *Fusarium* in cereals has been reviewed in detail by Chelkowski,[13] and there are many reports of the occurrence of zearalenone, with or without other *Fusarium* toxins, in cereals.[25, 59, 73] Zearalenone can be formed in the field (up to 21 mg/kg),[55] but the largest concentrations are usually associated with poor storage postharvest. Concentrations as high as 2.9 g/kg have been reported.[46] A survey of Canadian beers for *Fusarium* mycotoxins failed to demonstrate the occurrence of zearalenone,[66] and it seems unlikely that this metabolite will occur at significant levels in fermented milk products.

Trichothecenes

The trichothecenes form a very large family of sesquiterpene metabolites, the most important of which, in the context of foods, are produced by species of *Fusarium*. The terrible outbreaks of alimentary toxic aleukia in humans, and of hemorrhagic moldy corn toxicosis in farm animals, are especially associated with T-2 toxin (Figure 5–7a), which is produced primarily by *F. sporotrichioides* and *F. poae*. This is one of a group of very toxic trichothecenes that fortunately are relatively rare in crops grown for human consumption. The conditions leading to outbreaks of alimentary toxic aleukia are hopefully avoidable (i.e., widespread famine following a war). A much more common trichothecene is deoxynivalenol (Figure 5–7b), which is formed by *F. graminearum*, *F. culmorum,* and related species. Indeed, there are some years when samples from crops such as barley may show 100% incidence of deoxynivalenol (DON) because these species of *Fusarium* are patho-

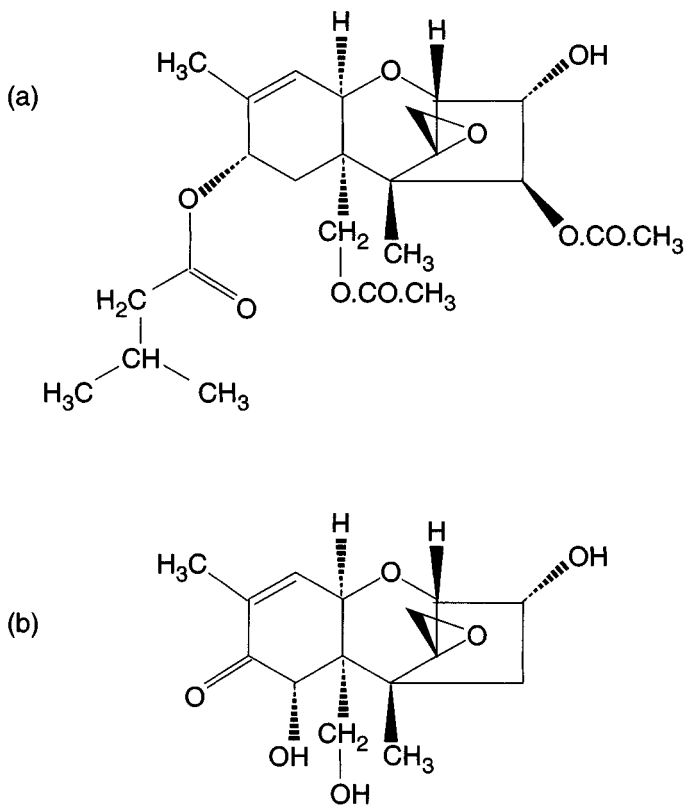

Figure 5–7 (a) T-2 toxin (b) Deoxynivalenol.

gens that may establish themselves in the field, causing red scab diseases, and continue their activity postharvest.

During the period 1996–1998, DON was widespread in cereals throughout the temperate parts of the world, with incidences from 43% to 100% and concentrations from 2 μg/kg to 62,050 μg/kg.[59] There is a clear account of the type of weather conditions that occurred during the growing season for maize in Maryland and Delaware during 1994, which led to DON contamination prior to harvest.[77] Although not as toxic as T-2 toxin, DON, like all the trichothecenes, is immunosuppressive and biologically active at concentrations very much less than the LD_{50} of 50–70 mg/kg. DON is a stable molecule and may pass into fermented cereal products, but it does not appear to be a problem in fermented dairy products. It would be surprising if DON were not found in beers and, indeed, it has been reported that 29 of 50 samples of Canadian and imported beer analyzed contained DON.[66] Although the majority of beers had very low concentrations of DON, nine had more than 5 ng/ml, and a single sample had as much as 50.3 ng/ml.

Fumonisins

The fumonisins are produced by *F. moniliforme* and related fusaria that do not produce trichothecenes. Fumonisin B_1 (Figure 5–8) is known to cause equine encephalomalacia, pulmonary edema in pigs, and a number of other illnesses in a range of animal species. It may be associated with esophageal carcinoma in humans[62] and has recently been the subject of a monograph.[28] An assessment of human exposure to the fumonisins for people in the Netherlands with a special emphasis on those people with gluten intolerance who would be especially at risk has been carried out.[18] The fumonisins are associated with maize and maize products, reflecting the host specificity of the molds producing it. In those commodities, it is remarkably widespread and can occur in relatively high concentrations. In his extensive review, Pittet[59] provided documentation of surveys in many countries during the period 1995–1998 with incidence of occurrence ranging from 20% to 100% and concentrations from 10 μg/kg to 37,650 μg/kg. These surveys included maize products such as polenta, corn flakes, and popcorn.

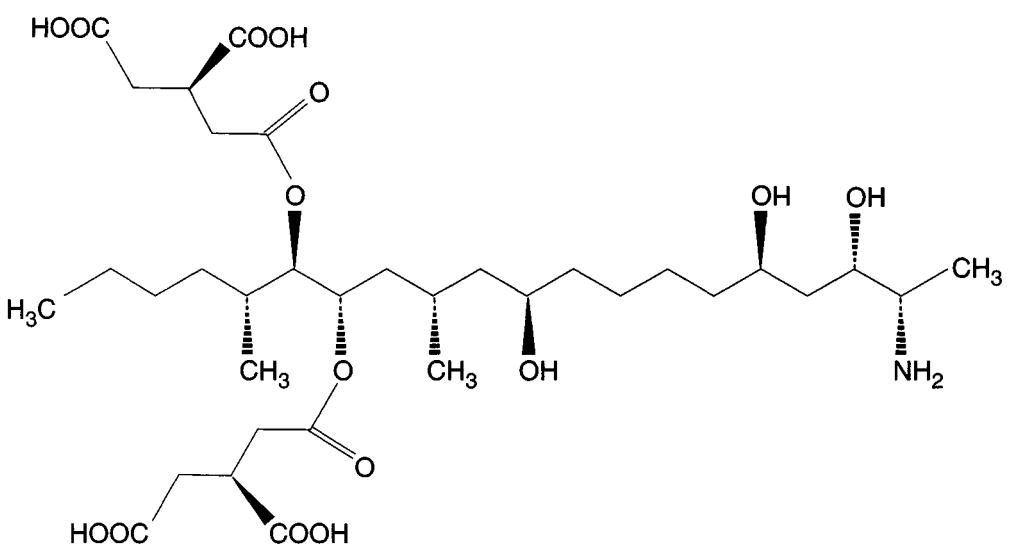

Figure 5–8 Fumonisin B_1.

As with DON, it would seem likely that fumonisins will be found in beers in which maize products are used at some stage during the manufacture, but there is no evidence of transmission from animal feeds to milk and hence to milk products. By using immunoaffinity columns for extraction and cleanup, it has been shown that 86% of samples of beer that was purchased in retail outlets in Lincoln, Nebraska, contained fumonisin B_1.[24] The Lincoln study followed an earlier report of fumonisin contamination of beer in Canada that had included beers that were imported from the United States.[67,70] These authors agreed that the most likely source of fumonisin in beer is the maize grits that are used as a brewing adjunct.

The fumonisins are relatively stable to elevated temperatures and survive a range of cooking, baking, and frying processes. Thus, after baking corn muffins spiked with fumonisin B_1 for 20 minutes at 175 or 200 °C, as much as 84% and 72%, respectively, survived.[29] A significant reduction when spiked corn masa was fried at 140–170 °C for up to six minutes was also found. Even frying contaminated chips for 15 minutes at 190 °C only reduced the fumonisin level by 67%. However, it has also been reported that, during extrusion cooking of corn grits, the reduction of fumonisin B_1 depended on several factors, including moisture content and whether or not mixing screws were used.[12] One of the problems in assessing the significance of the breakdown of the fumonisins is that they are esters of an aminopentol compound, and some food processes lead to the hydrolysis of the ester groups to the parent compound, which is still toxic.

The preparation of tortillas in Central and South America requires the treatment of maize with lime to produce nixtamal before cooking. Scott & Lawrence[68] validated the methodology for measuring the aminopentol formed from fumonisin B_1 in calcium hydroxide-processed foods such as tortilla and nacho chips, taco shells, and air-dried corn tortillas. They found significant levels of the aminopentol, but always at lower levels than the fumonisin B_1 from which it was derived. This implies that the latter had partly survived the alkaline process. Tortillas from villages in Guatemala may have as much as 185 mg kg^{-1} of AP_1 (i.e., the aminopentol derived from the hydrolysis of fumonisin B_1) and also still contain up to 10 mg kg^{-1} of fumonisin B_1 itself.[42] AP_1 may not always be formed during cooking and food processing of contaminated maize, even if fumonisin levels are reduced.[57]

ALTERNARIA TOXINS

Species of *Alternaria* cause dry and soft black rots of a range of commercially important crops and are often associated with the postharvest spoilage of fruits and vegetables.[58] The most common species is *A. alternata,* which can produce a number of toxic metabolites, the most important of which is tenuazonic acid (Figure 5–9). Other toxins include alternariol and its monomethyl ether, altenuene, and the altertoxins. The analysis of such a complex mixture requires a multitoxin method.[51] One or more of these toxins have been isolated from a number of cereals, oilseed rape, tomatoes, sunflower seed, and olives. There is the potential for these toxins to occur in the products of cereals, oilseeds such as sunflower, fruit juices, and tomato products, but there is no information regarding the effects of food processing on the survival of *Alternaria* toxins into fermented foods. Although isolates of *Alternaria* have been reported as part of the mold flora of some cheeses,[64] it is unlikely that *Alternaria* toxins will have any significance for human health from fermented foods. A detailed account of *Alternaria* species and their toxins is given by Bottalico & Logrieco.[6]

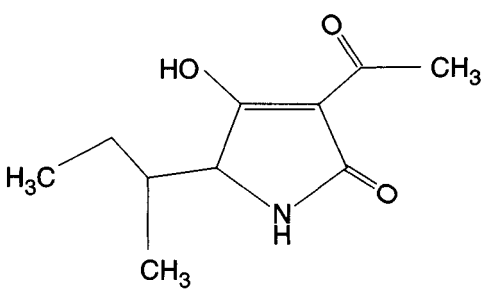

Figure 5–9 Tenuazonic acid.

BACTERIAL AND ALGAL TOXINS

The conditions for the growth of most food poisoning bacteria are generally well understood, and the control of growth can be effected by heat treatment, refrigeration, reduced A_w and low or high pH, alone or in combination. One or more of these parameters can also be combined with the presence of both synthetic or natural growth inhibitors. However, once bacterial toxins have been formed and released into the food, they are generally resistant to the conditions that inhibit or kill the producing organism.

Botulinum toxins are relatively large polypeptides (i.e., molecular weights of approximately 150 kDa) that are usually complexed with proteins as a progenitor toxin when they are released into the food. Such complexes are more stable at elevated temperatures and low pH than the pure neurotoxins. Of 14 outbreaks of botulism associated with dairy products between 1912 and 1997, the majority involved cheese or cheese spreads.[3,14,60] An especially serious outbreak in the United Kingdom during 1989 involving 27 cases and one death was a result of the consumption of hazelnut yogurt that was contaminated with type B neurotoxin produced by a proteolytic strain of *C. botulinum*.[54] The problem was not with the yogurt itself, which has a pH too low for growth and toxin production, but with the canned hazelnut conserve that was added to it. This was occasioned by a change in production from a product with a high sucrose content receiving a mild heat treatment to a product in which sucrose was replaced with aspartame, in response to a perceived public demand for low sugar products. The heat treatment remained unchanged and was insufficient to kill *C. botulinum* spores, but the high sucrose content prevented growth in the original formulation. The elevated A_w of the new formulation allowed growth and toxin formation in the hazelnut conserve and, although the pH of the yogurt to which it was added prevented further growth of the organism, it did not inactivate the toxin that had already been produced.

The enterotoxins of *S. aureus* are small single polypeptide chain proteins (i.e., molecular weights 26 to 30 kDa) that are more heat resistant than the producing organism. Although fermentation processes would normally prevent growth of this organism, there have been instances of enterotoxin production in cheese when the starter culture failed to grow fast enough,[80] and in salami sausage when a change in production conditions again allowed *S. aureus* to outcompete the organisms used for the fermentation.[53,74]

The emetic toxin of *B. cereus* known as cereulide is a cyclic dodecadepsipeptide[1] that is stable to proteolytic enzymes and remarkably heat stable (i.e., no loss of activity after 90 minutes at 121 °C). Cereulide is usually produced in cooked rice, pasta, and noodles, and is very unlikely to occur in fermented foods.

Bongkrek Poisoning

An Indonesian food known as tempeh bongkrek is made by inoculating coconut presscake with the mold *Rhizopus oligosporus*. Contamination of this food by the pseudomonad *B. cocovenenans*, previously known as *P. cocovenenans*,[81] unfortunately is not uncommon and has been responsible for quite a number of deaths. *B. cocovenenans* produces two toxic metabolites, toxoflavin (Figure 5–10) and bongkrekic acid (Figure 5–11), which has been shown to be a heptenetrioic acid.[16] Normally, the rapid growth of *R. oligosporus* over and into the solid substrate effectively inhibits bacterial growth, although bonkrekic acid formation is not necessarily directly correlated with bacterial numbers.

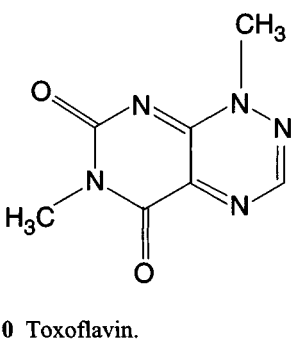

Figure 5–10 Toxoflavin.

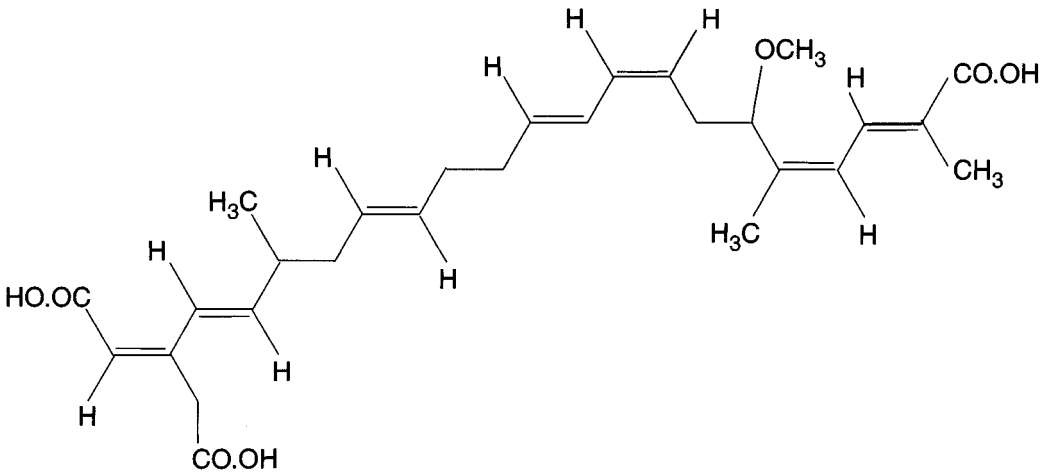

Figure 5–11 Bongkrekic acid.

Outbreaks of bonkrek poisoning seem to occur most often when coconut presscake is mixed with excessive amounts of coconut milk. The presence of both an aqueous environment and elevated levels of lipids seems to encourage bacterial growth and bongkrekic acid formation. It seems that temperature, pH, moisture, and salt concentration themselves do not fully explain why some tempeh bonkrek becomes toxic and another factor, such as the presence of appropriate lipids, may be implicated. In a useful review of the conditions influencing the formation of these toxins, it was specifically demonstrated that fatty acids have an important role in the formation of bongkrekic acid.[22] Although comparable in acute toxicity, bongkrekic acid is usually present at much higher concentrations than toxoflavin and is most likely to be of greatest significance in causing illness. A few hours after consumption of toxic tempeh bongkrek, people complain of malaise, abdominal pains, dizziness, sweating, and fatigue. Coma and death can follow within 20 hours of the onset of symptoms. The edible jelly fungus (*Tremella fuciformis*), which is cultivated in several countries in southeast Asia, is contaminated frequently with *B. cocovenenans*, and has also been implicated in bongkrek poisoning.

Cyanobacterial Toxins

A number of species of freshwater cyanobacteria belonging to the genera *Microcystis*, *Anabaena,* and *Aphanizomenon* can form extensive blooms in standing water and may cause deaths of animals drinking the contaminated water.[11] Cyanoginosin (Figure 5–12), a toxic metabolite of *Microcystis aeruginosa*, is an hepatotoxin. It is unlikely that these toxins will find their way into fermented foods, although there may be some concern that they may contaminate samples of *Spirulina* that are collected from the wild and used as food and health products.

Algal Toxins

Among the eukaryotic algae, it is the dinoflagellates and a small group of diatoms that cause concern because of their ability to produce very potent toxins. A number of toxic responses to contamination of seafoods occur, of which paralytic shellfish poisoning, diarrheal shellfish poisoning, and neurotoxic shellfish poisoning are all associated with marine dinoflagellates, and amnesic shellfish poisoning with species of marine diatom. Table 5–3 lists a selection of the

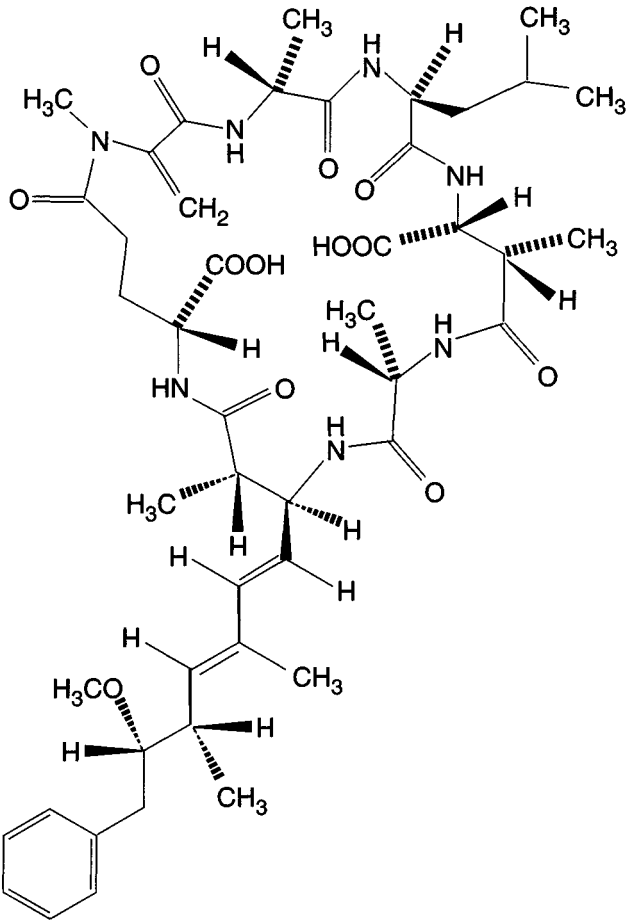

Figure 5–12 Cyanoginosin.

Table 5–3 A Selection of Eukaryote Algal Toxicoses

Toxicosis	*Associated Species*	*Toxins*
	Dinoflagellates	
Paralytic shellfish poisoning (PSP)	*Alexandrium catanella*	Saxitoxin (Figure 5–13)
Diarrheal shellfish poisoning (DSP)	*Dinophysis fortii*	Okadaic acid (Figure 5–14)
Neurotoxic shellfish poisoning (NSP)	*Gymnodium breve*	Brevitoxin
Ciguatera poisoning	*Gambierdiscus toxicus*	Ciguatoxin
	Diatoms	
Amnesic shellfish poisoning (ASP)	*Pseudonitzschia pungens*	Domoic acid (Figure 5–15)

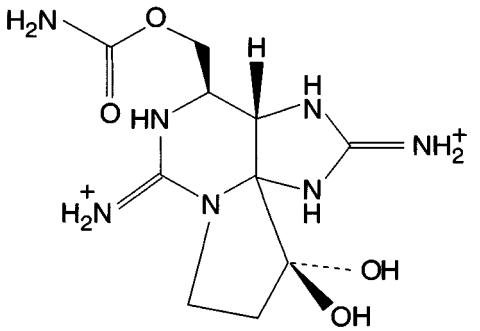

Figure 5–13 Saxitoxin.

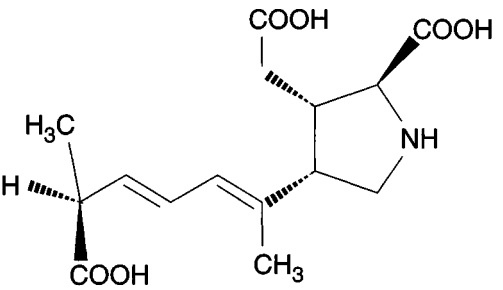

Figure 5–15 Domoic acid.

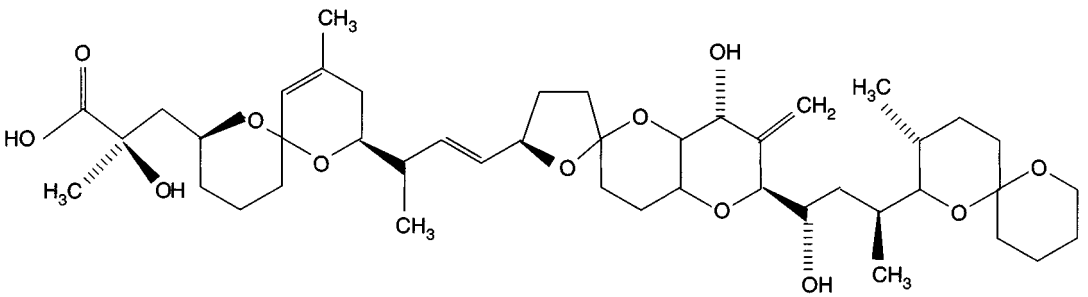

Figure 5–14 Okadaic acid.

species and toxins involved[27] (Figure 5–13, 5–14, and 5–15). These toxins are generally resistant to the temperatures involved in cooking and are likely to be unaffected by the salting and acidification that is associated with fermented fish products. In any case, it seems unlikely that the species of shellfish and fish involved will be used in the production of any fermented foods.

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