Mycotoxins in Food

Edited by

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Foreword

The original idea for this book was conceived in 1980 by Dr Deryck S. P. Patterson, Central Veterinary Laboratory, Weybridge, UK, who designed the outline of the book and selected most of the contributing authors. His untimely death in 1981 prevented him from fulfilling the task. This book is dedicated to the memory of Deryck Patterson and his impressive contributions to the mycotoxin research field.

PALLE KROGH

Contents

1 Introduction

P. KROGH

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An association between mycotoxins, i.e. toxic metabolites from microscopic fungi (moulds), and foodstuffs was already recognized when the discovery was made that disease in farm animals and humans could be caused by toxic moulds. Thus outbreaks of ATA in USSR in the 1930s were found to be causally associated with consumption of wheat grain, invaded by *Fusarium* moulds (Sarkisov, 1954), years later shown to produce trichothecenes (Mirocha and Pathre, 1973). Also, outbreaks of mass mortality in turkeys in the UK in 1960 were found to be linked to the ingestion of imported Brazil nut meal containing mycelium of a toxic mould, *Aspergillus flavus*, later shown to produce the "aflatoxins" (toxic and carcinogenic metabolites acting on the liver) (Goldblatt, 1969). Thus, the concept of foods being contaminated by mycotoxins was coined early in the history of mycotoxicology. As a consequence, when implementing control measures, countries all over the world have conducted surveys for specified mycotoxins in foodstuffs, particularly grains and oil seeds. Emphasis on food surveys is apparent in most monographs on mycotoxins published in the last two decades.

Previous monographs have covered the chemistry and biosynthesis of mycotoxins in foodstuffs, mycology of foodborne fungi, and toxicology of mycotoxins, based mainly on effects in animals, both experimental animals and episodes of field cases in domestic animals.

This monograph takes a different approach. After updating the information on the general aspects of mycotoxins, such as mycology, occurrence of mycotoxins, and toxicology, with an emphasis on the other food components acting in conjunction with mycotoxins, it makes an attempt to assess the significance of mycotoxins in the causation of disease in humans. In doing so, three groups of mycotoxins, aflatoxins, ochratoxins, and trichothecenes, have been singled out and treated in separate chapters, because these are the mycotoxins about which most is known in terms of human exposure.

Most episodes of human disease associated with mycotoxins are characterized by chronic manifestations, which follow exposure to mycotoxins

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over an extensive period of time. Analytical epidemiology provides the most convincing evidence in assessing the role of factors in disease causation. Investigations using analytical epidemiology are based on a statistical comparison of disease manifestations in which exposure to certain factors is measured quantitatively. So far only a few studies using analytical epidemiology have been conducted on the human diseases thought to be causally associated with mycotoxins, and all of the studies suffer from inadequacies in their design (Krogh, 1984). The main problem is the difficulty of measuring individual mycotoxin exposure, due to extreme variation in mycotoxin contamination, in terms of year-to-year, district-to-district and crop-to-crop variation. This variation is primarily a product of environmental factors, such as rainfall, as well as the harvest and storage conditions used. As a consequence of this variation, data from surveys of food commodities provide inadequate parameters for individual past exposure or predicted exposure, and no statistical model for epidemiological analysis has yet been devised to solve this problem.

In spite of the paucity of hard facts on the involvement of aflatoxins in human disease, toxicological and epidemiological data suggest that this group of mycotoxins is causally involved in the development of primary liver cancers. Further, aflatoxin may act as a co-factor with hepatitis B virus, one of the main risk factors (Wagstaff, 1985). It has been speculated that synergism between aflatoxin and hepatitis B virus is involved in the causation of primary liver cancer (Rajagoplan *et al.*, 1986; Ayoola, 1984), but so far no definitive proof of this hypothesis has been presented. There are other examples of possible interaction between aflatoxin and viable disease agents. Thus aflatoxin-induced liver carcinogenesis was found to be enhanced in rats concomitantly infected by a malaria-inducing agent, *Plasmodium berghei* (Angsubhakorn *et al.*, 1986), whereas the resistance of mice to the carcinogenic effect of aflatoxin was enhanced by concomitant infection with the liver parasite *Schistosoma mansomi* (Hasler *et al.*, 1986). Most epidemiological studies of mycotoxin-associated diseases in humans have considered only one factor, i.e. a particular mycotoxin, per disease. If the above mentioned viable agents are in operation also in human diseases, and considering the effect of dietary non-viable factors on the mycotoxin response, much more comprehensive epidemiological studies are warranted, dealing with a large number of risk factors, in order to assess the causal role of an individual mycotoxin.

Although humans are mainly exposed to mycotoxins by eating them in food, they may be exposed in other ways. Thus, aflatoxin has repeatedly been found in airborne dust from workplaces where aflatoxin-contaminated food commodities have been handled (Sorenson *et al.*, 1981; 1984). Lung diseases in humans, including lung cancer, have been encountered, associated with inhalation of air-borne aflatoxin (Baxter *et al.*, 1981; Dvorackova and Pichova, 1986). Occupational exposure to mycotoxins via the respiratory tract is thus an additional risk factor to be considered.

2 Food Mycology

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I YEASTS AND MOULDS

The fungi are a widely heterogeneous group of organisms which have in common a eukaryotic structure, a heterotrophic metabolism and a well-defined wall enclosing the cytoplasm. Several groups of fungi, the chytrids, the hyphochytrids and the oomycetes, are associated with aquatic habitats, a way of life reflected by the production of motile spores at some stage in their life cycles. The food mycologist is, however, mainly concerned with those fungi associated with terrestrial habitats, conveniently referred to as yeasts and moulds.

A The yeasts

The yeasts are essentially single-celled fungi which grow by fission or by budding (Fig. 1) and, although many species are important agents of food spoilage and several are used in food production, they are not known to produce mycotoxins. A few species are pathogenic to endothermic animals and may cause disease in humans. The best known is *Candida albicans*, which is carried as a harmless commensal by many people, but which may flare up as an infection of the throat, the vagina or even as a superinfection of the gastrointestinal tract, especially following oral antibiotic therapy. *Filobasidiella neoformans* Kwon-Chung, with its anamorphic state *Cryptococcus neoformans* (Sanfelice) Vuillemin, may cause a pulmonary infection leading to an often fatal meningoencephalitis. This heterothallic organism only produces mycelia and basidia when two compatible mating types come together. The asexual stage does not produce mycelia at all but a mass of subglobose to globose budding cells which are readily distinguished from other yeasts by the presence of a polysaccharide capsule. The organism is most readily isolated from bird droppings, particularly those of pigeons, but it may cause an infection in a very wide range of mammals, including humans. A distinct but related species, *Filobasidiella bacillispora* Kwon-

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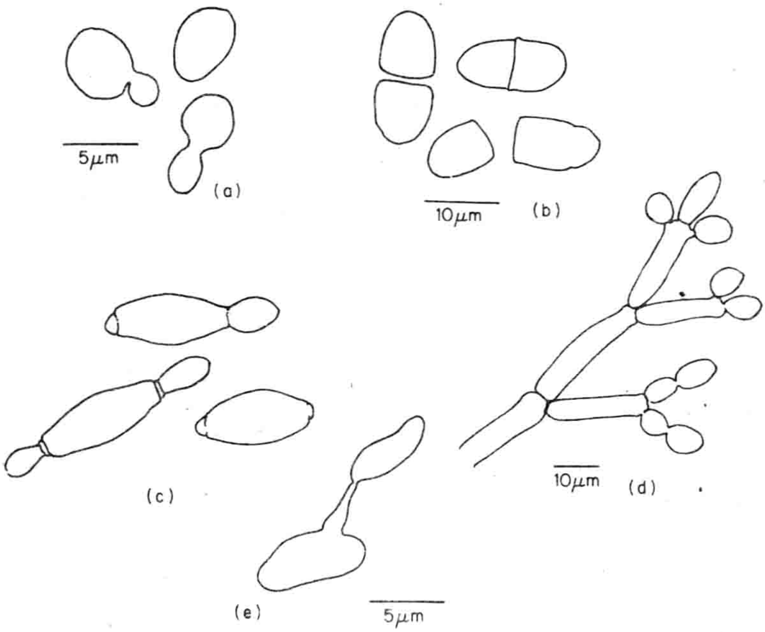

Fig. 1 Asexual reproduction of the yeasts. (a) Budding as in *Saccharomyces*. (b) Fission as in *Schizosaccharomyces*. (c) Bipolar budding as in *Saccharomycodes*. (d) The formation of pseudomycelium as in *Candida*. (e) Ballistospore formation as in *Sporobolomyces*.

Chung, causes cryptococcosis in Southern California and rarely elsewhere (Kwon-Chung, 1980).

Although the term "yeast", based on a dominant morphological character, is a convenient one, there are a number of fungi which are firmly accepted as yeasts but which frequently produce pseudomycelia, or even true mycelia. Indeed, there are some moulds, such as *Mucor rouxii* (Calmette) Wehmer, which are capable of growing with a yeast-like morphology. An interesting discussion of the relationships between yeasts and related filamentous fungi is given by Von Arx (1979). An indication of some of these relationships is shown in Table 1, from which it can be seen that the filamentous mould *Dipodascus*, and its anamorphic state *Geotrichum*, are considered to be related to the true yeasts.

B The moulds

The filamentous habit of the moulds is ideally suited to growth over surfaces and through solid substrates. The mycelium forms an efficient system for locally utilizing the available nutrients and transporting them to the actively growing hyphal tips. As the mycelium develops, branching and anastomosis

Table 1 Some relationships between yeasts and yeast-like moulds. (Adapted from Von Arx, 1979.)

			Examples of genera	
Class	Order	Family	Teleomorph	Anamorph
Hemiascomycetes	Endomycetales	Dipodascaceae	*Dipodascus*	*Geotrichum*
		Ascoidiaceae	*Saccharomycopsis*	*Candida*
		Saccharomycodaceae	*Hanseniaspora*	*Kloekera*
		Saccharomycetaceae	*Saccharomyces*	*Torulopsis*
		Metschnikowiaceae	*Metschnikowia*	*Candida*
Ustomycetes	Sporobolomycetales	Sporobolomycetaceae	*Aessosporon*	*Sporobolomyces*
Hymenomycetes	Aphyllophorales	Filobasidiaceae	*Filobasidiella*	*Cryptococcus*

occur, giving rise to a complex network of hyphae in intimate contact with the substrate. A great deal is now understood about the dynamics of hyphal growth and the mechanisms involved in the controlled production of the fungal wall (Burnett and Trinci, 1979), but of most importance from the point of view of food spoilage is that the mycelium provides a large surface area relative to the biomass, through which macromolecules, such as hydrolytic enzymes, and secondary metabolites, such as mycotoxins, may be secreted into the surrounding medium or substrate.

The major groups of filamentous fungi are shown in Table 2, with examples which may be important in food mycology. The classes of filamentous fungi are based on the nature of sexual reproduction and its end products (oospores, zygospores, ascospores or basidiospores, Fig. 2), and there is a separate class for the imperfect fungi. This last group contains a very heterogeneous assemblage of form genera, some individual species of which have been shown, on the basis of sexual reproduction, to be the anamorphs of one or other of the major classes.

1 THE LOWER FUNGI

Although the oomycetes are mainly aquatic fungi, some members of the group have become adapted to a more terrestrial habitat and, indeed, among the plant pathogenic members of the Peronosporales, there is even the development of airborne sporangia (Fig. 3). These terrestrial oomycetes require high relative humidities for growth and dispersal and will not normally cause any post-harvest problems in the storage of food, although plant tissue damaged by them in the field may be more susceptible to attack by other moulds during storage.

Among the oomycetes, *Saprolegnia diclina* Humphrey (=*Saprolegnia parasitica* Coker) is associated with a disease of fresh-water fish, and there are many species parasitic on plants of which the best known must be *Phytophthora infestans* (Mont.) de Bary, the causative agent of potato blight. Although there is evidence of high molecular weight phytotoxins being produced by species of *Phytophthora* (Ballio *et al.*, 1972; Paxton, 1972), the oomycetes are not implicated in any known mycotoxicoses.

The zygomycetes, particularly those of the Mucorales (Fig. 4), are much more clearly associated with a terrestrial habitat but, although a number of species of *Mucor, Absidia* and *Rhizopus* have been implicated in mycoses of animals (Landau and Newcomer, 1962), there are no clear indications of mycotoxin production by these moulds. *Absidia repens* Van Tiegham and *Mucor griseocyanus* Hagem have been reported to reduce aflatoxin B_1 to aflatoxicol (Detroy and Hesseltine, 1969) but it is unclear what significance such reactions might have in the natural history of mycotoxicoses. The zygomycetes require a higher water activity (a_w) for growth than the moulds producing aflatoxin, and their presence would indicate a very poor-quality

Table 2 The major groups of filamentous fungi of interest in food mycology. (Based on Ainsworth *et al.*, 1971.)

Subdivision	Class	Order	Example	Comments
Mastigomycotina	Oomycetes	Saprolegniales	*Saprolegnia parasitica*	Disease of fish
		Peronosporales	*Phytophthora infestans*	Potato blight
Zygomycotina	Zygomycetes	Mucorales	*Mucor* spp., *Rhizopus* spp.	Food spoilage
Ascomycotina	Plectomycetes	Erysiphales	*Erysiphe* spp.	Plant parasites
		Eurotiales	*Eurotium* spp.	Spoilage at low a_w
	Pyrenomycetes	Clavicipitales	*Claviceps purpurea*	Ergotism
		Hypocreales	*Nectria* spp.	Plant pathogens and mycotoxins
			Gibberella spp.	Plant pathogens and mycotoxins
		Sphaeriales	*Chaetomium* spp.	"Field" fungi, mycotoxins
	Discomycetes	Tuberales	*Tuber melanosporum*	Edible truffles
		Pezizales	*Morchella esculenta*	Edible
			Helvella and *Gyromitra*	Poisonous
Ustomycotina	Ustomycetes	Ustilaginales[a]	*Ustilago* spp.	Smuts, plant parasites
Basidiomycotina	Teliomycetes	Uredinales	*Puccinia* spp.	Rusts, plant parasites
	Hymenomycetes	Agaricales	*Agaricus campestris*, *Amanita phalloides*	Edible and poisonous toadstools
		Aphyllophorales	*Fistulina hepatica*	An edible example of a group which includes bracket fungi, fairy clubs and many others
Deuteromycotina (the fungi imperfecti)			*Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Pithomyces*, *Trichoderma*, *Alternaria*	Many mould genera, including mycotoxin producers

[a] This order is included in the Teliomycetes in Ainsworth *et al.* (1971) and by many other authors, but is placed in the Ustomycetes by Von Arx (1979) based on the separation of the smuts from the Basidiomycotina by Moore (1972).

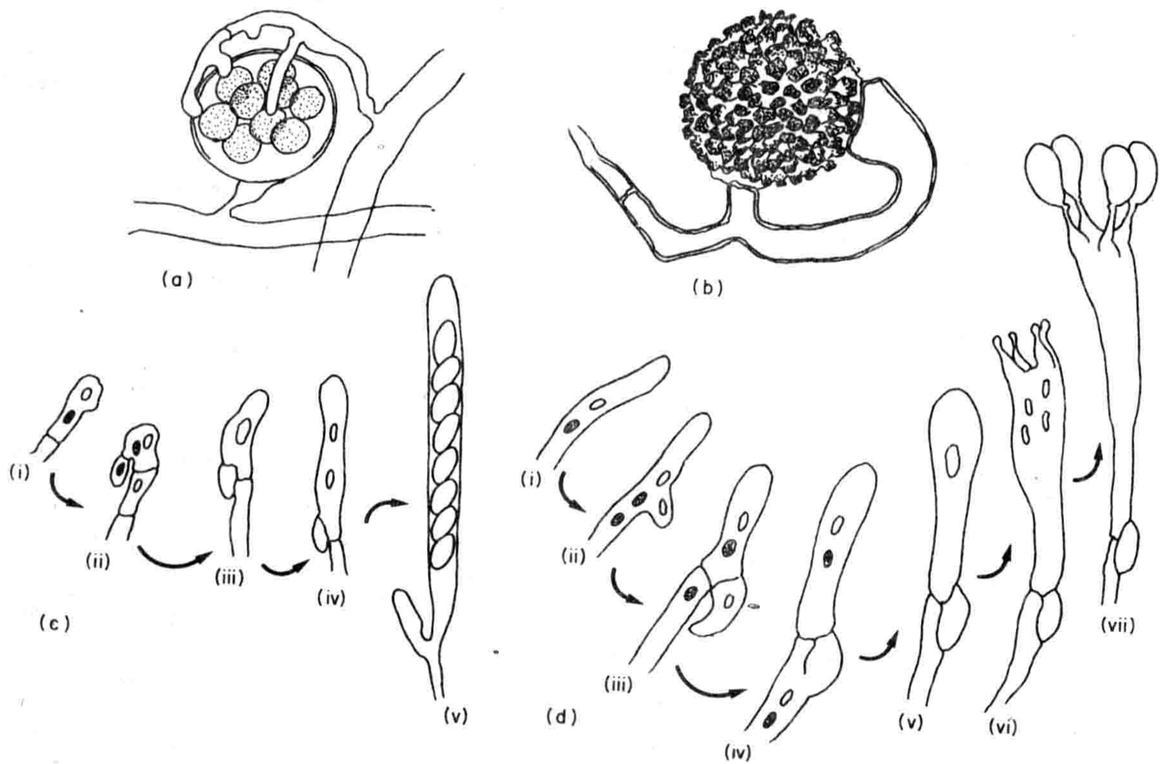

Fig. 2

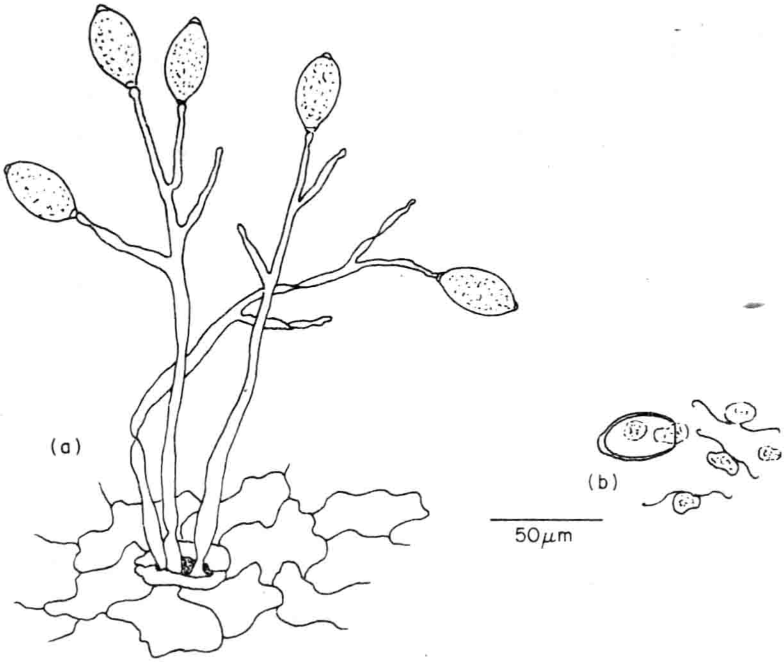

Fig. 3 Sporangium development in the oomycete, *Phytophthora infestans*. Sporangia are produced on aerial hyphae (a) and may behave as effective conidia or germinate to produce zoospores (b).

feed. Although the spores of *Mucor racemosus* Fresenius are able to germinate at an a_w of 0.88, and those of *Rhizopus nigricans* Ehrenberg at an a_w of 0.9, neither species can grow actively at an a_w activity of less than 0.92. If foods achieve these water activities during storage, then they are susceptible to spoilage by members of the Zygomycetes, especially those of the

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Fig. 2 Sexual reproduction in filamentous fungi. (a) Oospore formation in the oomycetes (*Achlya*). (b) Zygospore formation in the zygomycetes (*Zygorhynchus*). (c) Ascospore formation in the ascomycetes: a dikaryotic cell (i) divides to form a crozier (ii) in which karyogamy occurs (iii) followed by meiosis (iv) and the formation of eight ascospores in the ascus (v). (d) Basidiospore formation in the basidiomycetes: the dikaryotic state (i) is maintained by division and migration of the two compatible nuclei (ii) with the formation of a clamp connection (iii, iv), the formation of a diploid nucleus (v), meiosis and migration of the four nuclei to the tips of sterigma (vi) and the formation of basidiospores (vii).

Fig. 4 Asexual reproduction in the Mucorales. (a) *Mucor plumbeus*, spores released by dehiscence of the sporangium. (b) *Rhizopus stolonifer*, spores may remain attached to the collapsed columella. (c) *Thamnidium elegans*, forming readily detachable sporangiola as well as a typical sporangium. (d) *Syncephalastrum* sp. merosporangia are produced on the swollen tip of the sporangiophore, each producing a row of spores which may be released intact; the resulting structure has a superficial resemblance to *Aspergillus*. (e) *Cunninghamella* sp.; each sporangiole now only contains a single spore and behaves just like a conidium.

genera *Mucor* and *Rhizopus*, whereas *Thamnidium elegans* Link is associated with a superficial spoilage of meat in cold storage and, indeed, may even be used for tenderizing meat that has become tough.

2 ASCOMYCETES

This group of fungi, characterized by the production of ascospores within an ascus as a result of meiosis following the fusion of two compatible haploid nuclei, is an enormous assemblage (Fig. 5). The ascus may be naked as it is in the Hemiascomycetes; enclosed in a partial or complete structure known as the cleistothecium, as it is in the Plectomycetes; enclosed in an osteolate structure, the perithecium, as it is in the Pyrenomycetes; or, as in the Discomycetes, form part of the surface tissue of a more or less disc-shaped