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Microbiological facts and fictions in grain storage

Ailsa D. Hocking
Food Science Australia, PO Box 52, North Ryde, NSW 1670

Abstract. The safety and integrity of the food supply are of paramount importance and are among the drivers of safe grain storage. Bacterial contamination of stored grains may be a significant consideration for users where grain will not undergo further processing that includes a microbiocidal step, but problems caused by moulds and mycotoxins are more usually associated with grain storage. With an increased knowledge of the origins of mycotoxins, we now understand that at least some mycotoxins in some commodities are formed either before harvest, or immediately after harvest. Mycotoxins can be formed during storage, but only if there is sufficient moisture. Fungal growth usually develops as a complex succession of species, beginning with the most drought-tolerant (xerophilic) species. The metabolic activity of these pioneer species raises the moisture content of the grain, which may allow growth of mycotoxigenic species and, ultimately, the formation of mycotoxins.

Introduction

Ultimately, most stored grain is destined to end up as either human or animal food. Food safety and quality are driving factors in the long-term storage of grain. In the food safety area, bacterial pathogens and mycotoxins must be considered. They are often used in specifications set by domestic and international buyers of Australian grain. Quality issues include spoilage due to mould growth, insect and mite infestation, and general hygiene issues such as protection from rodents and birds.

Bacterial contamination

The bacterial species that occur commonly on grain are generally non-pathogenic, though contamination with bacterial pathogens such as *Salmonella, Escherichia coli* and *Bacillus cereus* can occur. *Salmonella* and *E. coli* are enteric bacteria, and their presence on grain is usually an indication that it birds or rodents have contaminated it. This may occur during harvesting, but more often is a result of poor hygiene in road or rail trucks during transportation, or poor pest control during storage. Levels of contamination with enteric pathogens are usually very low.

Most grain destined for human food is first milled into flour or other grain products such as semolina, wheat germ and bran. The milling process may contribute to the microbiological load of the flour, but flour then usually undergoes further processing, such as baking, that will kill most bacteria. Conditioning wheat to increase the moisture content to a level suitable for milling can also increase the counts of bacteria, yeasts and moulds (Berghofer et al. 2003). The microbial contaminants are concentrated in the outer grain layers (bran and wheat germ). These are removed during milling, leaving the end product, flour, relatively clean and usually pathogen-free. Enteric pathogens such as *Salmonella* are rarely isolated from straight run flour, although *B. cereus* may be present in low numbers (Berghofer et al. 2003).

Mycotoxin contamination before storage

The presence of mycotoxins in grain was traditionally regarded as an indicator of poor storage conditions. The corollary to this was that mouldy grain contained mycotoxins. Neither statement is necessarily true. Mycotoxins may be produced as a result of poor storage, but they may already be present in grain coming into storage. Conversely, not all moulds that grow in stored commodities produce mycotoxins.

Some of the fungi associated with grain in the field (often referred to as 'field fungi') can form mycotoxins, either immediately before, or just after harvest. *Alternaria, Fusarium, Aspergillus* and *Penicillium* can all act as pre- or postharvest pathogens of grain, and may form mycotoxins. *Alternaria* and *Fusarium* do not compete strongly at reduced water activities (a_w) so are unlikely to form mycotoxins once the grain is dry, or during storage. Conversely, *Aspergillus* and *Penicillium* are more often considered as 'storage fungi'. They are known to form mycotoxins in stored grains, and are usually not regarded as fungi that can produce mycotoxins before harvest.

Mycotoxins formed by *Fusarium*

Many *Fusarium* species are capable of forming mycotoxins in grains, but there are two species (and those closely related to them) that are regarded as having higher potential to cause mycotoxin contamination of grains: *F. graminearum* and *F. verticillioides* (previously known as *F. moniliforme*).

F. graminearum and the closely related species *F. culmorum* cause head scab or head blight in wheat, rye and triticale, and stalk and cob rot of maize (Burgess et al. 1994). Other small grain crops such as barley and oats may also be affected. *F. graminearum* is more common in wheat in warmer climates, such as Australia, North America and China, whereas *F. culmorum* is the dominant species in cooler growing areas such as Finland, France, Poland and The Netherlands (Miller et al. 2001). The most important mycotoxin formed by these *Fusarium* species is the trichothecene toxin, deoxynivalenol, also known as DON or vomitoxin (Miller et al. 2001).

F. verticillioides (= *F. moniliforme*) is widely distributed throughout the world, and appears to be established in maize wherever it is grown. It causes stalk and cob rot of maize, but also basal stalk rot and root rot of sorghum, foot rot of rice and crown rot of asparagus. *F. verticillioides* and closely related species such as *F. proliferatum* form fumonisins in maize and sorghum (Burgess et al. 1994). Equine species are particularly susceptible to this mycotoxin, which causes leucoencephalomalacia, also known as mouldy corn disease or blind staggers, in horses (Marasas et al. 2001).

Pre-storage contamination with aflatoxins

Some grains and oilseeds may be contaminated with aflatoxins before, or just after, harvest. Peanuts are particularly susceptible to aflatoxin contamination before harvest, and considerable research has been done on this problem in Australia. Peanut plants may be colonised during the growth cycle by strains of *Aspergillus flavus* or *Aspergillus parasiticus,* both of which are capable of producing aflatoxins (Pitt et al. 1991). Under dryland conditions, if the peanut plants experience stress (e.g. drought stress) close to harvest time, then aflatoxin may be formed in the maturing peanuts. Aflatoxin levels may also increase immediately after harvest during field drying, particularly if the in-field drying period is extended by rain.

Other commodities that may be colonised by *A. flavus* before harvest, with consequent formation of aflatoxins, are maize, cottonseed and tree nuts such as pistachio and brazil nuts (Olsen 2003). Aflatoxins may affect many other commodities during storage, as *A. flavus* and related species grow well at reduced a_w (down to about 0.80), and aflatoxins may be produced down to approximately 0.85 a_w (Hocking and Pitt 2003).

Postharvest contamination with ochratoxin A

Ochratoxin A (OA) was first isolated from *Aspergillus ochraceus* in 1965, in a laboratory study searching for new toxic metabolites from moulds. OA was then found as a natural contaminant of maize in 1969 in the USA. At about the same time, studies were being conducted in Scandinavia on a kidney disease in pigs, which appeared to be related to mouldy feed. These studies showed that OA was the cause of the disease now known as porcine nephropathy. Since then, OA has been found as a contaminant of grains in most European countries, and in northern North America.

The source of OA in cooler climates is principally from contamination of small grains by *Penicillium verrucosum* (Pitt 1987). Investigations on farms in Denmark, Sweden and the UK have indicated that *P. verrucosum* contaminates grains such as wheat and barley during or immediately after harvest, and that the sources of contamination include the harvesting machinery, dryers and silos (Olsen 2003). Although *P. verrucosum* is relatively xerophilic, being capable of growth at 0.80 a_w (Pitt and Hocking 1997), OA production ceases below about 0.86 a_w (Northolt et al. 1979), so most OA contamination probably occurs immediately after harvest, while grain has not yet dried to a safe moisture content.

Other preformed mycotoxins

Grains are not the only crops that may be affected by mycotoxin contamination before harvest. Aflatoxins can be found in dried figs. The spores of *A. flavus* may contaminate the ripening figs through insect damage or wounds. Once the fruits begin to sun dry on the tree, the relatively warm temperatures and slightly reduced a_w favour the growth of *A. flavus,* with subsequent aflatoxin formation (Doster and Michailides 1998). Figs may also become contaminated with OA if *Aspergillus niger, A. carbonarius* or *A. ochraceus* colonise late in the ripening phase (Doster et al. 1996).

Grapes can become contaminated with OA, with consequent contamination of grape products (juice, wine, wine vinegar and dried grapes). The source of this contamination is growth of species of black *Aspergillus*, particularly *A. carbonarius,* just before harvest. Grapes are particularly susceptible if rain damage causes splitting and ring-necking of the ripe berries (Hocking et al. 2001).

Mould development and mycotoxin formation in stored grain

In Australia, grain is usually taken into storage at relatively low moisture content (less than 12.5%). If the a_w (or equilibrium relative humidity; ERH) of the grain remains below 0.60 (or 60% ERH), moulds are unable to grow,

and the stored grain will be stable. However, if temperature and moisture gradients develop in the store, localised pockets of higher moisture may develop, opening the way for mould germination and growth.

The moulds that start the deterioration process in stored grains can grow at low a_w and are known as xerophiles. Xerophilic moulds usually cannot be detected on freshly harvested grains, as they occur in very low numbers. The most likely contamination sources for xerophilic fungi are the storage silos or sheds themselves, but transport vehicles, dust etc. may also contribute to the contamination. As moulds grow and become established, their metabolic activities create microenvironments with elevated temperature and moisture content. As the moisture content increases, conditions become suitable for other, less xerophilic, moulds in a process known as fungal succession (Wicklow 1995) (Figure 1).

Aspergillus penicillioides and closely related species are often the pioneer species, as they are able to germinate and grow at a_w values near 0.66–0.67. As they become established and the moisture content increases to 0.68–0.70 a_w, common *Eurotium* species and *Wallemia* can begin to grow. Less xerophilic species can then develop, such as *Aspergillus candidus*, which as well as being moderately xerophilic, tolerates reduced oxygen conditions. Once the moisture content has increased to an equivalent of 0.80 to 0.82 a_w, mycotoxigenic species such as *A. flavus* (aflatoxins), *A. ochraceus* (ochratoxin), *A. versicolor* (sterigmatocystin), and some of the more xerophilic *Penicillium* species, may begin to develop (Frisvad 1995). Mycotoxin formation may start at this point, but many species are unable to produce mycotoxins at water activities below about 0.85. Temperature will also influence the fungal populations that develop, with *Aspergillus* species more common in warmer regions such as Asia and Australia, and *Penicillium* species developing in cooler climates such as northern North America and Europe (Frisvad 1995).

Mould growth and quality issues

Mould growth in grains may cause deleterious changes in addition to the formation of mycotoxins. Many spoilage fungi cause loss of germination in seed grains, discolouration and darkening of the grains, reduction in protein content, musty odours, and changes in fatty acid profiles and other constituents of the grains. Mould development may also encourage mite and insect infestation (Wicklow 1995).

Mould development during storage can be controlled or prevented by ensuring that grain is adequately dry at intake. Further protection can be provided by preventing the development of temperature and moisture gradients by cooling and/or aeration of the grain. Protection from insect infestation will also help prevent mould development in stored grains, both in bulk or bag storages.

Conclusions

Contamination of stored grain by bacterial pathogens is generally not a major issue, as pathogens such as *Salmonella* survive poorly on grain. Furthermore, most grain

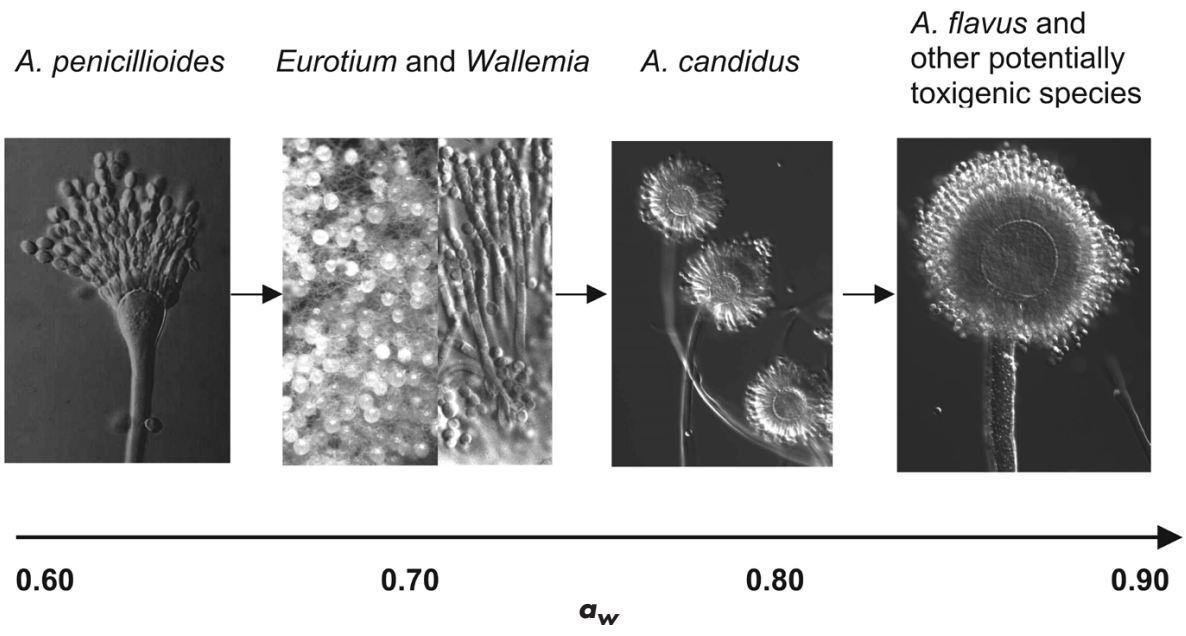

Figure 1. Schematic showing fungal succession in stored grain. *Aspergillus penicillioides* is a pioneer species that can germinate near 0.67 a_w. As its metabolic activities raise the local a_w to about 0.69–0.70 a_w, *Eurotium* and *Wallemia* can start to grow, further raising the available moisture to allow growth of other species such as *A. candidus* near 0.78–0.80 a_w, and eventually mycotoxigenic species including *A. flavus* and *Penicillium* species once the a_w reaches about 0.80–0.83.

destined for human food use will undergo processing steps that will destroy bacterial pathogens before commodity reaches the consumer.

Mycotoxin contamination of grain is not always a result of poor storage, as some mycotoxins, particularly *Fusarium* toxins, may already be in the grains when they are brought into storage. However, if moulds do start to grow in stored grains, mycotoxins may be formed if the fungal succession develops to a point where mycotoxigenic species are able to grow, generally above about 0.83 a_w. Whether or not mycotoxins are an issue, any mould development in stored grains is undesirable.

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