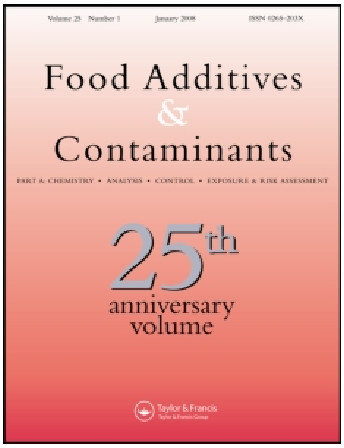

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Strategies to reduce mycotoxin levels in maize during storage: a review

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Strategies to reduce mycotoxin levels in maize during storage: a review

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Maize (*Zea mays* L.) is one of the main cereals as a source of food, forage and processed products for industry. World production is around 790 million tonnes of maize because as a staple food it provides more than one-third of the calories and proteins in some countries. Stored maize is a man-made ecosystem in which quality and nutritive changes occur because of interactions between physical, chemical and biological factors. Fungal spoilage and mycotoxin contamination are of major concern. *Aspergillus* and *Fusarium* species can infect maize pre-harvest, and mycotoxin contamination can increase if storage conditions are poorly managed. Prevention strategies to reduce the impact of mycotoxin in maize food and feed chains are based on using a hazard analysis critical control point systems (HACCP) approach. To reduce or prevent production of mycotoxins, drying should take place soon after harvest and as rapidly as feasible. The critical water content for safe storage corresponds to a water activity (a_w) of about 0.7. Problems in maintaining an adequately low a_w often occur in the tropics where high ambient humidity make the control of commodity moisture difficult. Damage grain is more prone to fungal invasion and, therefore, mycotoxin contamination. It is important to avoid damage before and during drying, and during storage. Drying maize on the cob before shelling is a very good practice. In storage, many insect species attack grain and the moisture that can accumulate from their activities provides ideal conditions for fungal activity. To avoid moisture and fungal contamination, it is essential that the numbers of insects in stored maize should be kept to a minimum. It is possible to control fungal growth in stored commodities by controlled atmospheres, preservatives or natural inhibitors. Studies using antioxidants, essential oils under different conditions of a_w, and temperature and controlled atmospheres have been evaluated as possible strategies for the reduction of fungal growth and mycotoxin (aflatoxins and fumonisins) in stored maize, but the cost of these treatments is likely to remain prohibitive for large-scale use.

Keywords: mycology; mycotoxins; fungi; cereals

Introduction

Maize (*Zea mays* L.) is an important source of food, forage and processed products for industry. Global production during 2008 reached around 790 million tonnes. Today, there is a dichotomy in the world through the demands of the First World for quality food uncontaminated with chemical residues and the desperate need of Third World populations to protect their agricultural products from damage by insect pests and fungal contamination in order to maintain a minimum level of food safety.

Stored maize is a man-made ecosystem in which quality and nutritive changes occur because of the interactions among physical, chemical and biological factors.

Fungal spoilage and mycotoxins contamination are of major concern. Fungal infection can result in mycotoxin contamination during growing, harvesting, storage, transport and processing. The main fungal species and mycotoxins associated with maize are *Aspergillus flavus* and aflatoxins, *Fusarium verticillioides* and *F. proliferatum* and fumonisins, *F. graminearum* and trichothecenes and zearalenone. *A. flavus* can infect maize pre- and post-harvest and an increase in aflatoxin content can occur if the phases of drying and storage are poorly managed. Although *Fusarium* species are predominantly considered as field fungi, it has been reported that fumonisin production can occur post-harvest when storage conditions are inadequate (Marin et al. 2004). Both aflatoxins and fumonisins are relevant in maize and maize-based foods and feeds due to their widespread occurrence and co-occurrence.

In Argentina maize is normally harvested with a water content between 14% and 16% ($a_w = 0.72$–0.80), but sometimes maize can be harvested with a high moisture content of 18–20% ($a_w = 0.90$–0.93), so that to reduce or prevent production of most mycotoxins drying should take place as soon after harvest and as rapidly as feasible. The critical water content for safe

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storage corresponds to a water activity of 0.70 (Magan and Aldred 2007).

In storage many insect pests can attack maize grains, among them *Sitophilus zeamais* Mots, *Rhyzopertha dominica* F., and *Tribolium castaneum* Herbst (Rozado et al. 2008). Moisture can accumulate from their activities and this situation provides ideal conditions for fungal proliferation and mycotoxin accumulation. In order to reduce the impact of mycotoxin in the food and feed chains, it is necessary to control insect pests and fungal contamination.

Insect pests control

The severe attack of insect pests during grain storage is responsible for substantial losses in the grain storage sector all over the world. Prevention of insect pests have been achieved mainly by a chemical strategy but the intensive use of chemical compounds has resulted in the evolution of resistant populations and presence of chemical residues both in foods and in the environment.

Phosphine is an effective fumigant for disinfection of storage maize and other commodities in most developed countries after the loss of methyl bromide (Bell 2000).

Essential oils offer an alternative to phosphine to control insect pests. Contact and fumigant insecticidal actions have been demonstrated for a range of essential oil constituents.

In the last few years, the demand for alternatives pest-control technologies for use in stored grains opens new areas of research, and the application of ozone is attracting attention because of its advantages. It can be generated electrically on site at the time of use, eliminating the need to store and dispose pesticide packages, and it leaves no residue (Kells et al. 2001; Pereira et al. 2007; Sousa et al. 2008).

Another promising alternative to the use of conventional pesticides in stored product protection is the use of diatomaceous earth (DE). These compounds are the fossilized remains of diatoms, have low mammalian toxicity, and act on the insects in a physical manner (Subramanyam and Roesli 2000). An enhanced mixture of DE with the plant extract bitterbarkomycin (BBM) showed activity on the adults of insect pests of stored grains of maize, wheat and barley at doses of 50, 100 and 150 $mg\,kg^{-1}$ (Vayias and Stephou 2009).

Chemical synthetic and natural antifungal agents

Organic acids

Weak acids are used in food and feed to prevent fungal spoilage. The most common weak acid preservatives used are sorbic acid, benzoic acid, and propionic acid. These compounds are fungistats and show antimicrobial activity only when they are present as undissociated acids. The efficiency of these acids, therefore, depends on the dissociation constant, pK_a. Also, special care needs to be taken in order to guarantee the efficient coverage of the grains. It was shown that mycotoxigenic fungi can sometime metabolize these preservatives. *F. verticillioides* and *F. proliferatum* growth and fumonisin production were not affected by a mixture of propionic or sorbic acids (Marin et al. 2000).

Antioxidants

The fungicidal and fungistatic activities of food-grade antioxidants, e.g. butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl paraben (PP) controlling growth, and aflatoxin synthesis by *Aspergillus flavus* and *A. parasiticus*, have been observed (Thompson 1991, 1992).

In *in vitro* experiments, BHA, PP and BHA/PP mixtures had a negative impact on the mycoflora and aflatoxin accumulation in maize grains (Nesci et al. 2003). In stored maize it was observed that BHA and PP at a concentration of 20 $nmol\,l^{-1}$ affected the mycoflora and *Aspergillus* section *Flavi* populations (Nesci et al. 2008).

Butylated hydroxyanisol (BHA) and propyl paraben (PP) controlled both growth and fumonisin production by *F. verticillioides* and *F. proliferatum in vitro* on culture media and in irradiated maize under different conditions of water activity and temperature (Etcheverry et al. 2002; Torres et al. 2003). These antioxidants at doses of 500 and 1000 $\mu g\,g^{-1}$ were effective in controlling the growth of *Penicillium*, *Aspergillus* and *Fusarium* populations, and fumonisin production under different water activity conditions (0.98–0.95) on unsterilized maize during 28 days of incubation. The reduction in fumonisin accumulation varied between 32% and 77%, being the maximum reduction achieved with BHA at 1000 $\mu g\,g^{-1}$ after 28 days. The reduction in fumonisin levels could be explained by the combined effect of antioxidants and the competing mycoflora (Farnochi et al. 2005).

Another study showed that using low concentrations of two antioxidants may have a synergistic inhibitory effect on growth and fumonisin production by *F. verticillioides* and *F. proliferatum* (Reynoso et al. 2002).

The effect of antioxidants BHA and resveratrol and an extract of *Lentinus edodes* on sterilized and non-sterilized maize seeds at $0.95a_w$ inoculated with *F. graminaerum* showed that resveratrol at 230 and 23 $mg\,kg^{-1}$ and BHA 0.02% had a significant effect (>80%) on fungal growth and toxin production. The levels of ZEA production in the unsterilized maize grains was lower compared with those found in the sterilized one (Ricelli et al. 2005).

Marin et al. (2006) evaluated the effect of synthetic *trans*-resveratrol and RES VIN®, a sub-product of vineries on fumonisin and ZEA production in maize grains. No effect in fumonisin production was observed, but a reduction in ZEA production was observed when *trans*-resveratrol or RES VIN were added.

Due to widespread public concern for long-term health and the environmental effects of synthetic pesticides in developed counties, natural pesticides of both microbial or plant origin are being evaluated in order to control fungal spoilage and mycotoxins production in different storage grains including maize.

Investigations in several countries confirm that some plant essential oils have fungicidal actions against toxigenic fungi (Solisman and Bodeaa 2002; Velluti et al. 2003).

The effect of essential oils from 41 vegetable species from Argentina on the growth of *Aspergillus* section *Flavi* was evaluated. Clove, mountain thyme and poleo showed the best activity (Bluma et al. 2008). The antifungal activity of *Pimpinella anisum* L. (anise), *Pëumus boldus* Mol. (boldus), *Hedeoma multiflora* Benth. (mountain thyme), Syzygium *aromaticum* L. (clove), and *Lippia turbinate* var. intergrifolia (griseb) (poleo) essential oils against *Aspergillus* section *Flavi* growth and aflatoxin production was evaluated in sterile maize grain under different conditions of water activity. The essential oils showed an effect on growth rate and aflatoxin accumulation. Their activity was dependent on water availability, concentration and time of incubation. Compositional analysis of the essential oils showed that carvacrol, α-*p*-cimene, terpinolene, anethol, and eugenol were the main components present in the different essential oils evaluated (Bluma and Etcheverry 2008). The effects of eugenol on growth and mycotoxins production by toxigenic fungal genera like *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. have also been reported (Bullerman et al. 1977; Cairns and Magan 2003; Velluti et al. 2003; Hope et al. 2005).

Essential oils can be a practical application, and the advantage is their bioactivity in the vapour phase, a characteristic that makes them attractive as a fumigant for stored product protection, such as maize grains (Tripati and Dubey 2004).

Compounds derived from ligninolitic fungi (*Lentinula edodes* and *Trametes versicolor*) showed inhibition of aflatoxin production (Table 1). Antimicrobial and antioxidants compounds, such as thioproline, manitol, and α- and β-glucans, can be responsible for the modulation of oxidative stress and toxin production (Fanelli et al. 2000; Reverberi et al. 2007; Zjalic et al. 2006).

Table 1. Effect of the lyophilized filtrates (2%) of *Trametes versicolor* and *Lentinula edodes* on aflatoxin production on sterilized maize seeds inoculated with *A. parasiticus* conidia.

	Aflatoxin (μg 20 g^{-1})	Aflatoxin inhibition (%)
Control	109.6 ± 6.9	0
L. edodes CF 21	36.6 ± 2.4	66.6 ± 5.8
L. edodes CF 24	28.4 ± 1.6	74.1 ± 5.2
L. edodes CF 42	9.8 ± 0.4	91.1 ± 6.4
T. versicolor CF 74	31.6 ± 2.0	71.1 ± 6.3
T. versicolor CF 76	24.2 ± 1.7	77.9 ± 5.5
T. versicolor CF 117	3.4 ± 0.2	96.9 ± 6.9

Note: Data are the mean ± standard error (SE) of three determinations of three separate experiments.
Sources: Ricelli et al. (2005), and Zjalic et al. (2006).

A correlation between lipoperoxide formation in cells of *A. parasiticus* and aflatoxin biosynthesis has been observed both in culture media and on maize seeds. The relation appears to be driven by activation of certain oxidative stress-related transcription factors such as yap 1-like, skn 7-like and hsf 2-like. Activation of these factors promoted the transcription of genes encoding antioxidants-related enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) (Kim et al. 2006; Reverberi et al. 2008).

The effectiveness of different compounds such as antioxidants BHA, PP alone or mixtures of BHA and PP, essential oils, and fungal extracts has been demonstrated at both laboratory scale or pilot scale, but their use in natural grains in storage facilities is limited due to the cost of treatment. For example, it has been demonstrated that BHA (100–200 ppm), PP (200 ppm), and resveratrol (23–230 ppm) gave >90% reduction in fumonisin, ZEA, and DON accumulation in maize. The cost of using synthetic resveratrol (Sigma-Aldrich, Dorset, UK) in comparison with RES VIN (a by-product of the wine industry) and BHA, K sorbate and Na propionate by kilogram of treated seed have been estimated from around €149 to €0.44 and €0.12, respectively (S. Marin et al., personal communication). The possibility of using industrial-grade antioxidants like RES VIN or binary or tertiary mixtures of industrial-grade parabens could offer more economic sources to control mycotoxins production in stored products like maize, but the cost of these compounds is prohibitive for large-scale use.

Modified atmospheres for mycotoxin prevention in stored maize grains

In maize grain ecosystems, the most important abiotic conditions that influence growth and mycotoxins production are a_w, temperature, and gas composition (Magan et al. 2004).

Fungal species involved in the deterioration of stored maize are obligate aerobes, but they can grow under conditions of reduced levels of oxygen, and some species can tolerate high levels of CO_2. The tolerance to low O_2 and high CO_2 concentrations is influenced by the availability of water.

The effect of modified atmospheres in controlling fungal growth and mycotoxin production in stored products has been reported by Dixon and Kell (1989), Ellis et al. (1993), and Magan and Lacey (1988). Studies on modified atmospheres with different CO_2 levels balanced with O_2 and N_2 showed that *A. flavus* grew on wheat and rye with up to 75% CO_2 (Suhr and Nielsen 2005).

Aflatoxin production was also evaluated on maize grain during storage for up 21 days at 25°C under $a_w = 0.95$ and 0.92. Up to 75% CO_2 resulted in an inhibition of the *A. flavus* populations in the grains. The efficacy of controlled atmospheres Xa_w showed that treatment with 25% CO_2 reduced *A. flavus* development, but at least 50% CO_2 was necessary to reduce aflatoxin synthesis (Giorni et al. 2008).

Samapundo et al. (2007) showed that fumonisin production by *F. verticillioides* and *F. proliferatum* was inhibited by 30% CO_2 at 0.985a_w, by about 10–20% at 0.951a_w, and by 10% at 0.93a_w. The CO_2 concentrations were obtained as initial values in sealed systems.

Although modified atmosphere storage is used for control of both moulds and insects in moist stored grains, sometimes it has been observed that regimes sufficient for controlling fungal growth are not effective to control storage insect pests that survive and grow over a wider equilibrium humidity range. At present, more studies, mainly pilot scale, are necessary to determine the possibility of using controlled atmospheres for controlling mycotoxins production in stored maize grains.

Hermetic temporary storage: silo-bags

In developing countries the permanent production increase is not met by the increase of permanent storage facilities, so that silo-bags are used. Due to their low cost, their use is increasing among farmers to store maize and other commodities (Bartosik et al. 2008).

Each silo-bag can hold approximately 180–200 tonnes. These plastic bags are 60 m long, 2.74 m diameter, and the plastic cover is made of three layers (white outside and black inside), of 235 µm thickness. The silo-bags are waterproof and have a certain degree of gas-tightness (O_2 and CO_2). The CO_2 and O_2 concentrations in the silo-bag depend on the balance between respiration, the entrance of external O_2 to the system, and the lost of CO_2 to the ambient air (Figure 1).

Pacin et al. (2009) evaluated fumonisin contamination in maize stored in silo-bags. They showed that fumonisin contamination in maize stored following good agricultural practices increased significantly.

Further studies are needed to evaluate this practice on fungal and mycotoxins contamination on maize grains by adding synthetic or natural antioxidants.

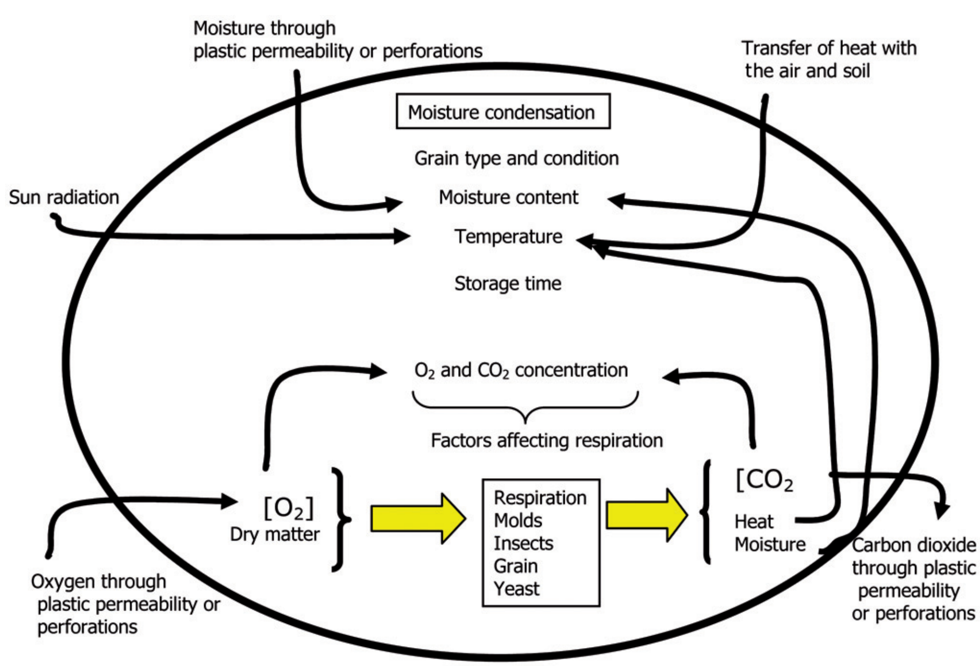

Figure 1. The main factors affecting the respiration of grain and microorganisms in the silo-bag, the relationships among them, and the final O_2 and CO_2 concentrations.
Source: Cardoso et al. (2008).

Ozone

Ozone has also been evaluated to control fungal populations in stored maize. Kells et al. (2001) showed that 50 mg kg^{-1} of ozone reduced the cfu g^{-1} of *Aspergillus parasiticus* in stored maize. The use of ozone as a strategy to control toxigenic fungi and mycotoxins production in stored maize needs further evaluations.

Conclusions

The accumulation of Mycotoxins in crops is increasing worldwide due to climatic changes, the use of plant varieties of high yield but which are susceptible to mycotoxin accumulation, and agricultural practices. Some mycotoxins can contaminate maize before harvest and their levels can increase during the post-harvest stages of the food and feed chains since the stored maize agroecosystem is a complex one in which interrelation may occur between biotic and abiotic factors.

Whether mycotoxins are labelled a biological or a chemical hazard, they fit in an HACCP programme, and appropriate critical control points and their critical limits must be identified. Good agricultural practices are the foundations on which HACCP is based (Aldred et al. 2004). The *FAO/IAEA Manual on the Application of HACCP System in Mycotoxin Prevention and Control* (Food and Agricultural Organization (FAO) 2001) provides examples in which the HACCP system is applied to mycotoxin contamination in food and feed in developing country scenarios. Different strategies have been evaluated to reduce the entry of mycotoxins in stored maize. Prevention strategies are based on using the HACCP approach and identifying the critical control points. There are key management aspects to consider: the efficient and prompt drying of maize for medium- and long-term storage in hygienic silos free of insect pest and fungal populations; and accurate and regular moisture content measurement to ensure safe thresholds are not breached.

Synthetic antioxidants have been shown to be effective in controlling the accumulation of mycotoxins in maize, but the use of alternative compounds is being evaluated such as essential oils and fungal extracts. However, there are yet many economic and technological hurdles associated with this type of approach.

Industrial-grade antioxidants have a good efficacy as an analytical grade (Marin et al. 2006; Passone et al. 2007). These findings suggest that these compounds may have applications in commodities destined for animal feed, but the cost of these compounds is likely to remain prohibitive for large-scale use.

Modified atmospheres using carbon dioxide can reduce the toxin accumulation in maize, but the concentration needed is too high to reach in storage facilities.

The use of ozone to control insect pests and fungal populations in maize offers a promising tool, considering the advantages of this gas: it can be produced *in situ*, it has low cost, the its only by-product is oxygen. The effect of ozone on toxin production needs further study.

For temporary hermetic storage, a silo-bag is a low-cost alternative for storing maize in farms when permanent facilities are not available.

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