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The influence of modified atmospheres and their interaction with water activity on the radial growth and fumonisin B_1 production of *Fusarium verticillioides* and *F. proliferatum* on corn. Part I: The effect of initial headspace carbon dioxide concentration

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Abstract

The effect of modified atmospheres on the growth and fumonisin B_1 production of *Fusarium verticillioides* and *Fusarium proliferatum* on corn is presented in a series of two papers. In this, the first part, the effect of initial headspace (IH) carbon dioxide concentration and its interaction with water activity (a_w) on growth and fumonisin B_1 production was evaluated. It was observed that at all a_w values studied, increase in the IH CO_2 concentration generally resulted in a decrease in the colony growth rate (g, mm day^{-1}) and maximum colony diameter (D_{max}, mm) and an increase in the lag phase duration (λ, day). Although both a_w and IH CO_2 concentration had significant and synergistic effects on g, a_w had the largest effect. As little as 10% IH CO_2 completely inhibited the production of fumonisin B_1 by *F. verticillioides*. *F. proliferatum* was more resistant and required 40, 30 and 10% IH CO_2 at a_w 0.984, 0.951 and 0.930, respectively, to completely inhibit fumonisin B_1 production. These results demonstrate that modified atmospheres containing high CO_2 levels could potentially be employed for the protection of corn from fungal spoilage and mycotoxin contamination during the post-harvest period.

Keywords: *Fusarium verticillioides*; *Fusarium proliferatum*; Modified atmospheres; Headspace carbon dioxide; Water activity; Fumonisin B_1

1. Introduction

In the field and during storage, corn is attacked by a number of fungal species that are of great economic and health importance (CAST, 2003). Of these, the *Fusarium* spp. have gained much interest ever since the more recent isolation and elucidation of the structure of the fumonisins (Gelderblom et al., 1988). Of importance to societies subsistent on corn as a staple food product is the epidemiological association of fumonisins or *Fusarium* contaminated corn with esophageal cancer in many regions worldwide including Northern Italy (Franceschi et al., 1990), Transkei region of South Africa (Thiel et al., 1992), the Linxhian region of China (Chu and Li, 1994) and South-eastern United States (Gelderblom et al., 1992). The International Agency for Research on Cancer currently classifies fumonisin B_1 as a Group 2B compound (possibly carcinogenic to humans) (IARC, 1993).

Although the *Fusarium* spp. are predominantly considered as field fungi (Magan and Lacey, 1988), Pelhate (1968) suggested that they should be considered as an intermediate group between field and storage fungi as they can develop in stored moist grain. Marin et al. (2004) also reported that fumonisin production may occur post-harvest when the storage conditions are inadequate. Moreover, Bacon and Williamson (1992) reported that the Fusaria are one of the most prevalent fungal species associated with maize worldwide both pre- and post-harvest. The contamination by *Fusarium* spp. is often an additive process, which begins in the field and potentially

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increases during harvest, drying and storage (CAST, 2003). Their presence in stored grain has been found to significantly decrease the quality, nutritional and economic value of harvested grain (Bottalico et al., 1989; Marin et al., 1998).

Several approaches have been investigated to inhibit fungal growth and mycotoxin production on corn. Novel pre-harvest strategies evaluated to control fungal growth on the field include the use of endophytic bacterium such as *Bacillus mojavensis* and *Bacillus subtilis* as biological control agents (Bacon and Hinton, 2000; Bacon et al., 2001), the introduction of non-mycotoxigenic strains and the use of cultivars less susceptible to infection (CAST, 2003). Post-harvest strategies have mainly been based on the application of chemical antifungal agents such as sorbates, propionates, benzoates (Punja and Grogan, 1982), butylated hydroxyanisole (Ahmad and Bruanen, 1981) and bicarbonate salts (Montville and Shih, 1991).

In the last decade controlled atmospheres (CA) and modified atmosphere packaging (MAP) have successfully been applied as preservation techniques. This success has largely been a result of their ability to extend the shelf-life whilst maintaining the natural quality of food products and satisfying the ever rising consumer demand for foods free of chemical residues (Jayas and Jeyamkondan, 2002). As moulds are facultative aerobes and are highly sensitive to CO_2 (Smith et al., 1990; Farber, 1991), a potential exists for MAP to replace the conventional use of chemical agents to protect bulk stored grain systems from potential fungal growth and mycotoxin production. Despite this potential, to date most studies on stored grains have rather been focused on the ability of MAP to control insect pests (Emecki et al., 2002). A useful review of these studies is discussed by Jayas and Jeyamkondan (2002). The success of modified or controlled atmospheres in eradicating insect pests indirectly reduces the possibility of fungal contamination as most insect pests are fungal vectors. The ability of MAP to inhibit fungal growth and mycotoxin production on food products normally stored in bulk has been reported by Diener and Davis (1972), Wilson et al. (1975, 1977), Magan and Lacey (1984, 1988) and Ellis et al. (1993).

Most of the studies of the effect of MAP on fungal growth and mycotoxin production are on *Aspergillus flavus* and aflatoxins (Paster and Bullerman, 1988), resulting in a paucity of data on other moulds including fumonisin producing Fusaria. The only reports available on fumonisin producing *Fusarium* species include Wilson et al. (1975) and Gibb and Walsh (1980) who investigated the ability of *Fusarium verticillioides* to survive on corn in high CO_2 atmospheres, whereas Musser and Plattner (1997) investigated fumonisin production under anaerobic conditions in sealed bags. Most of these studies have also been performed at one a_w value/moisture content value and therefore very few reports exist on the possible interaction of the effects of modified atmospheres and a_w. The major objective of this study was to address this paucity by investigating the effects of initial headspace (IH) CO_2 or O_2 concentrations and their interaction with a_w on growth and fumonisin B_1 production of the two major fumonisin producing *Fusarium* spp. on corn. This first part focuses on the effects of the IH CO_2 concentration and its interaction with a_w.

2. Materials and methods

2.1. Fungal isolates

F. verticillioides Sheldon (25 N) and *Fusarium proliferatum* (Matsushima) Nirenberg (73 N) were obtained courtesy of the Food Technology Department Culture Collection of the University of Lleida, Spain. The isolates were maintained at 7 °C on potato dextrose agar (PDA) (Oxoid, Basington, UK).

2.2. Growth substrate

Dried yellow dent corn supplied by Aveve Belgium (NV) was used as growth substrate. The corn had an initial moisture content of 12.79±0.45 kg/100 kg dry matter (kg/100 kg dm) and a_w of 0.698±0.015. The corn was sterilized by 25 kGy of γ-irradiation at IBA Mediris (Fleurus, Belgium) to eliminate any fungal contamination whilst maintaining the natural ability of the grain to germinate. The sterilized corn was stored aseptically at 7 °C until use.

2.3. Experimental design

A full factorial combination of six levels of IH CO_2 (0, 10, 20, 30, 40 and 60%) and three a_w values (0.93, 0.95 and 0.98) was also used to investigate the effect of CO_2 concentration on growth and fumonisin B_1 production of both isolates at 25 °C. All conditions had an IH O_2 concentration of 20±1% O_2 with N_2 making up the balance of the gas.

2.4. Preparation of growth substrate and inoculum

Adsorption isotherms developed at 25 °C (Samapundo et al., 2007) for the corn were used to determine the amount of sterile distilled water that had to be aseptically added to the irradiated corn to reproducibly achieve the desired a_w values. After addition of water, the grain was equilibrated at 4 °C over two days with periodic mixing before it was transferred to an incubator at 25 °C for a further day. This allowed the growth substrate to achieve the same temperature as that of final incubation, which ensured constant pre-inoculation conditions. The final a_w was confirmed by a Novasina Thermoconstanter TH200 (Axair Ltd. Systems, Pfapfikkon, Switzerland).

The inoculum was prepared by using a sterile inoculation loop to scrape off sporulating mycelia from the surface of PDA sub-culture slants. These were then transferred to the centre of petri plates containing PDA and incubated at 30 °C for 6 days. They were then transferred to 25 °C for a further 24 h which enabled the isolates to adapt to the final incubation temperature.

2.5. Inoculation of the corn, packaging and incubation

Approximately 24 g of rehydrated corn was aseptically weighed into each petri plate. Using a sterile cork borer, a 5 mm diameter disk was cut from the margin of the seven day old colony on PDA and transferred to the centre of the petri plates containing sterile hydrated corn. Four inoculated plates were