Available online at www.sciencedirect.com
ScienceDirect

Applied Radiation and Isotopes 65 (2007) 927–933

Applied Radiation and Isotopes

www.elsevier.com/locate/apradiso

Effects of gamma radiation on maize samples contaminated with *Fusarium verticillioides*

F.L. Ferreira-Castro[a], S. Aquino[b], R. Greiner[c], D.H.B. Ribeiro[a], T.A. Reis[a], B. Corrêa[a,*]

[a]*Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes 1374, CEP 05508-900 São Paulo, Brazil*
[b]*Instituto de Pesquisas Energéticas e Nucleares, Centro de Tecnologia das Radiações, Av. Prof. Lineu Prestes 2242, CEP 05508-000 São Paulo, Brazil*
[c]*Federal Research Centre for Nutrition and Food, Centre for Molecular Biology, Haid-und-Neu-Strasse 9, D 76131 Karlsruhe, Germany*

Received 28 July 2006; received in revised form 15 March 2007; accepted 21 March 2007

Abstract

The efficacy of γ-irradiation as a method of decontamination of maize containing *Fusarium verticillioides* under controlled conditions of relative humidity (RH) (97.5%) and water activity has been studied. Maize grains inoculated with a spore suspension of *F. verticillioides* were irradiated to 2, 5, and 10 kGy. Thereafter, the irradiated and control samples were analyzed for the presence of fumonisins, their viable cells were counted, and their morphology was investigated by electronic microscopy. It was found possible to decrease the risk of exposure to fumonisins by irradiating maize to 5 or 10 kGy. However, at the dose of 2 kGy, the survived fungi (36%) can produce more fumonisins than the fungi in the control unirradiated samples under the same conditions.

Keywords: Maize; Decontamination; Sterilization; *Fusarium verticillioides*; Gamma radiation; Fumonisins.

1. Introduction

Mycotoxins are toxic metabolites affecting the health of humans and animals. Although the effects of fumonisins on humans are not well understood, occurrence of these compounds in maize was associated with high incidences of esophageal and liver cancers (Fandohan, 2003; Bullerman, 1996).

Mycotoxins are secondary metabolites produced primarily in the idiostage of fungal growth. Many of the food-borne filamentous fungi are capable of producing mycotoxins, and approximately 300 different mycotoxins have been identified. However, only 20 mycotoxins produced by various species are relevant to human health (Geisen, 1998). Fumonisins are produced by species of genera *Fusarium*, mainly *F. verticillioides* (*F. moniliforme*) and *F. proliferatum*, which are widespread in nature and are frequently isolated from maize and animal feed (Riley et al., 1998). Several studies have identified fumonisin B_1 as the principal cause of the neurotoxic syndrome equine leukoencephalomalacia in horses (Kellerman et al., 1990), hydrothorax and pulmonary edema in swine (Osweiler et al., 1992), hepatic cancer in mice (Gelderblom et al., 1991), as well as of the reduction of growth, heart diseases, immune deficiency, and degenerate necrosis of the liver in birds (Ledoux et al., 1992). Fumonisins have been documented to a lesser extent because they were discovered later than mycotoxins (Bullerman, 1996).

Ionizing radiation is widely used for preservation of food (Rustom, 1997). It destroys bacteria and molds that grow naturally in raw products utilized in food production. In addition to inactivating bacteria, molds and fungi, the radiation treatment increases the shelf life of fruit by changing maturation, germination and senescence, as well as by killing insects (OMS, 1989).

The Joint FAO/IAEA/WHO Expert Committee, which met in 1976 and 1980, concluded that irradiation is an appropriate physical process for treating foods. The committee stated that a maximum overall average dose of 10 kGy presents no toxicological hazard, and, hence,

---

*Corresponding author. Tel.: +55 11 3091 7295; fax: +55 11 3091 7354.
E-mail addresses: fabilucy@usp.br (F.L. Ferreira-Castro), correabe@usp.br (B. Corrêa).

0969-8043/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apradiso.2007.03.011

toxicological testing of the foods treated in this manner is no longer required. Furthermore, doses up to 10 kGy pose no particular nutritional or microbiological problems (WHO, 1977). Mycotoxin-contaminated commodities cannot be completely eliminated at present. The development and acceptance of a General Code of Practice by Codex will provide all nations with uniform guidelines on control and management of contamination by various mycotoxins. This Code of Practice will be effective only if the producers in each nation apply the general principles of the Code with due consideration of the peculiarities of the local crops, climate, and agronomic practices. It is important for the producers to realize that good agricultural practices (GAP) represent the primary line of defence against contamination of cereals with mycotoxins, which should be complemented by good manufacturing practices (GMP) during the handling, storage, processing, and distribution of cereals for human food and animal feed (Codex Alimentarius, 2003).

F. verticillioides is one of the predominant fungi in the corn intended for human and animal consumption worldwide. In view of this fact, the purpose of this work was to evaluate the effects of various doses of γ-radiation on *F. verticillioides* and fumonisin toxin in maize.

2. Materials and methods

2.1. Maize grains samples

The maize (*Zea mays* L.) simple hybrid semi-hard—BRS 1010 with a known fungal contamination and fumonisin content was used for the experiment in four groups: control, Group 1 (G1), Group 2 (G2), and Group 3 (G3). A 200-g sample was used in each group. The samples were individually packaged in plastic bags, wrapped in paper bags, and sealed. The samples were irradiated to 20 kGy to eliminate the natural fungi.

2.2. Humidity and temperature control

The samples were kept in sterile dishes inside a plastic container with a relative humidity (RH) of 97.5%. This RHrelative humidity was produced by 200 mL of a 30% solution of potassium sulfate (K_2SO_4), which provided the water activity (A_w) of 0.98 (Winston and Bates, 1960).

2.3. Inoculation of spore suspension in the maize samples

The spore suspension was prepared by diluting *F. verticillioides* strain (from the Programma on Mycotoxins and Experimental Carcinogenesis—PROMEC do National Research Institute for Nutritional Diseases, Medical Research Council, South Africa) in a phosphate buffer solution—PBS (NaCl, 8 g/L; KCl, 0.2 g/L; KH_2PO_4, 0.12 g/L; Na_2HPO_4, 0.9 g/L). After shaking, 100 mL of PBS was mixed with two drops of Tween 80. The spores were counted in a Neubauer chamber. The suspension concentration was adjusted to 1×10^6 spores/mL. Then, the 200-g corn samples were inoculated by spraying with 2 mL of the fungal spore suspension (using a pipette). The samples were kept in plastic containers, while humidity and temperature were controlled with a thermo-hygrometer. The containers were sealed and incubated at 25 °C for 15 days.

2.4. Irradiation

The samples were irradiated in a ^{60}Co γ-ray facility of the Instituto de Pesquisas Energéticas e Nucleares—IPEN/CNEN in São Paulo, Brazil. The radiation source was Gammacell 220 (MDS Nordion, Ottawa, Canada); the dose rate was 4.74 kGy/h, and the irradiation temperature varied between 25 and 28 °C. As noted previously, the test samples were given doses 20 kGy to eliminate the natural microbial contamination. A fungal suspension was inoculated to the corn samples and incubated for 15 days. The samples of Groups 1, 2, and 3 were given doses 2, 5, and 10 kGy, respectively. The control sample was not irradiated.

2.5. Plate counting

After the irradiation, 10-g aliquots of the samples were ground, thoroughly mixed with 90 mL of sterile distilled water and shaken in sterile tubes for 30 min. Spore counting was performed by the plate-count technique in the DRBC agar (OXOID) medium using each suspension in a serial dilution from 10^{-1} to 10^{-6}. After incubation at 25 °C for 7 days, the counting was performed according to Pitt et al. (1983), and the results are expressed in colony-forming units per gram of maize (cfu/g).

2.6. Fluorescent viability test

For a viability test, a 3-g portion of each sample (control, G1, G2, and G3) was suspended in 1 mL of distilled water. A 0.1 mL portion of this suspension was added to 0.1 mL of a solution containing fluorescein diacetate (FDA; 2 μg/mL) and ethidium bromide (EB; 50 μg/mL), both in the PBS buffer with pH 7.4. The mixture was put onto a slide for a fluorescent microscopy analysis. This mixture was incubated at 25 °C for 30 min, according to Corrêa et al. (1990).

2.7. Determination of fumonisins in the corn samples

According to a modified technique originally described by Sydenham et al. (1996), FB_1 and FB_2 were extracted from 10 g of ground sample with 100 mL of methanol/water (3:1) 28 h after the irradiation. The mixtures were shaken for 30 min and filtered through Whatman No. 1 paper. The SAX minicolumn (Varian Bond Elute cartridge containing 500 mg silica, with 10 mL capacity) was conditioned with 5 mL of methanol and 5 mL of methanol/water (3:1). After passing 10 mL of the filtered extract through the cartridge, we purified the sample with 5 mL of methanol/water (3:1) and, then, with 3 mL of methanol. The eluate obtained by elution with 10 mL of a mixture of methanol and acetic acid (99:1) was evaporated to dryness. After that, 100 μL of the sample was derivatized with 200 μL of an *o*-phthaldialdehyde solution (OPA; 40 mg of OPA, 1 mL of methanol, 5 mL of 0.1 M sodium tetraborate and 50 μL of 2-mercaptoethanol) for 2 min. FB_1 and FB_2 were detected by high-performance liquid chromatography (HPLC) using a Shimadzu SCL-6B chromatograph. Fumonisins were separated in a C-18 reverse-phase column (5 ODS-20, $l = 150$, $D = 4.6$ mm, Phenomenex). FB_1 and FB_2 derivatives were determined with a fluorescence detector (model RF10AX) at 335 and 440 nm (excitation and emission, respectively). A mixture of acetonitrile, water, and acetic acid (1:1:0.5) was used as a mobile phase; the flow rate was 1.0 mL/min, and the column temperature was 30 °C. This composition of the mixture provided adequate pressure and viscosity of the mobile phase, which resulted in optimal separation and retention times (8.4–8.9 min for FB_1 and 24.2–25.5 min for FB_2). The detection limit of the method was 50 μg/kg for both the compounds, and the recoveries were 86% for FB_1 and 87% for FB_2.

2.8. Scanning electron microscopy (SEM)

Three grains from each irradiated sample were examined by SEM. The grains had been stored in a 40% glutaraldehyde solution for 24 h and dried at 42 °C for at least 48 h. The dried grains were mounted on aluminum stubs, coated with gold, scanned, and photographed.

2.9. Water activity measurements

The water activity of the samples was determined in an AQUALAB CX-2 machine (DECAGON, Pullman, WA, USA). The moisture content of the samples was measured with a digital thermo-hygrometer (Digital Thermo-Hygro Clock).

2.10. Data analysis and statistical evaluation

The statistical analysis of the results was performed with the GraphPad StatMate program (Version 1.01i, GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1. Unirradiated maize

We have found that the total fungal counts varied in the range $(1.7–5.4) \times 10^5$ (Table 1). Among the isolated fungal genera (*Fusarium*, *Penicillium* e *Cladosporium*), *F. verticillioides* species dominated: their frequency varied between 14.29% and 90.0%.

Table 1
Relative frequencies of occurrence and the numbers of colony-forming units in the samples of unirradiated maize

Sample	Isolated fungi	Frequency of occurrence (%)	cfu/g
1	*F. verticillioides*	14.29	2.8×10^5
	Cladosporium sp.	67.86	
	Penicillium sp.	17.86	
2	*F. verticillioides*	90.00	3.0×10^5
	Penicillium sp.	10.00	
3	*F. verticillioides*	83.33	3.0×10^5
	Penicillium sp.	16.67	
4	*F. verticillioides*	47.06	1.7×10^5
	Penicillium sp.	47.06	
	Cladosporium sp.	5.88	
5	*F. verticillioides*	64.81	5.4×10^5
	Penicillium sp.	1.85	
	Cladosporium sp.	33.33	

Some authors in Brazil reported presence of *Fusarium* in cereal. Almeida et al. (2002), who analyzed the fungal microflora of maize samples from Capão Bonito and Ribeirão Preto cities of São Paulo State, reported presence of *F. verticillioides* in 35% of samples from Ribeirão Preto and 49% of samples from Capão Bonito. In a similar study, Orsi et al. (2000), who worked with maize samples of different hybrids, revealed predominance of genera *Fusarium*.

A level of water activity (A_w) above 0.87 is necessary for fungal growth, while A_w above 0.90 is essential for fumonisin production (Cahagnier et al., 1995; Lacey et al., 1991; Leitão, 1998). The water activities for the analyzed samples ranged between 0.83 and 0.86 (average of 4.34 mg/kg for FB_1 and 0.35 mg/kg for FB_2).

3.2. Irradiated maize

3.2.1. Fungal populations and concentrations of fumonisins

γ-Irradiation has effectively inhibited fungal growth and reduced the concentrations of fumonisins in Groups 1 and 2. The average control values were in the range $(3–15) \times 10^7$ cfu/g. Irradiation to 2 kGy (Group 1) reduced the average concentration to $(3–5.5) \times 10^4$ cfu/g. In Group 2 (irradiated to 5 kGy), no fungal growth was detected in one of the samples, but there were between 1×10^2 and 2×10^2 cfu/g in the others. The samples irradiated to 10 kGy (Group 3) were completely inactivated (Table 2). The percentages of viable spores found in Groups 2 and 3 were 6% and 0%, respectively. There have been many reports that radiation treatment is a suitable method for decontaminating foods. Aziz et al. (1997a, b) showed that the counts of viable florae decreased approximately exponentially with the radiation dose and the effective dose for the elimination of these microorganisms was about 5 kGy for all the medicinal plants used in their study.

Saleh et al. (1998) found species of *A. alternata*, *Cladosporium cladosporioides*, *Curvularia lunata*, and *C. geniculata* to be more resistant to γ-radiation among 10 species of fungi representing the genera *Alternaria*, *Cladosporium*, *Curvularia*, *Fusarium*, and *Penicillium*. Onyenekwe et al. (1997) investigated what dose would be sufficient for eliminating the natural microflora from a species of pepper (*Piper guineese*). They found a high incidence of *Fusarium* sp. in samples irradiated to 5 kGy and concluded that 10 kGy would be necessary to decontaminate the spices completely. Ramakrisna et al. (1991) eliminated microorganisms from wheat seeds entirely with 12 kGy.

Table 2
Numbers of colony-forming units per gram (cfu/g) in irradiated maize samples

cfu/g + LDL[a]	Log 10	Antilog
Control		
$15 \times 10^7 + 1$	8.1761	
$3 \times 10^7 + 1$	7.4771	
$11 \times 10^7 + 1$	8.0414	
$6 \times 10^7 + 1$	7.7782	
$8 \times 10^7 + 1$	7.9031	
Mean	7.8752	7.501×10^7
SD	0.2678	
Group 1		
$4.0 \times 10^4 + 1$	4.6021	
$5.5 \times 10^4 + 1$	4.7404	
$3.3 \times 10^4 + 1$	4.4771	
$5.0 \times 10^4 + 1$	4.6990	
$5.0 \times 10^4 + 1$	4.6990	
Mean	4.6435	4.4×10^4
SD	0.1060	
Group 2		
$2.0 \times 10^2 + 1$	2.3032	
$1.0 \times 10^2 + 1$	2.0043	
$0.0 \times 10^2 + 1$	0.0000	
$2.0 \times 10^2 + 1$	2.3032	
$1.0 \times 10^2 + 1$	2.0043	
Mean	1.7230	0.000519×10^5
SD	0.9747	
Group 3		
$0.0 \times 10^1 + 1$	0.0	
$0.0 \times 10^1 + 1$	0.0	
$0.0 \times 10^1 + 1$	0.0	
$0.0 \times 10^1 + 1$	0.0	
$0.0 \times 10^1 + 1$	0.0	
Mean	0.0	0.1×10^1
SD	0.0	

[a] LDL, lower detection limit (assumed to be 1 spore, $n = 5$).

The concentrations of fumonisins in our samples also changed as a result of the irradiation. The unirradiated samples in the control group had, on average, 6.4 mg/kg FB_1 and of 0.5 mg/kg FB_2. The concentrations of fumonisins found in the irradiated samples were inversely proportional to the radiation dose (Table 3). In Group 2, the levels of FB_1 were reduced approximately by 21%, and the levels of FB_2 remained unchanged. In Group 3, the levels of FB_1 were reduced approximately by 62.5%, and the levels of FB_2 roughly by 40%.

The concentrations in the Group 1 samples irradiated to 2 kGy (12.3 mg/kg for FB_1 and 0.8 mg/kg for FB_2) were higher than the concentrations in the control sample, which may be attributed to the size of the inoculum. The size of the inoculum affects toxin formation, as demonstrated with *A. parasiticus* (Sharma et al., 1980) and *A. flavus* (Odamtten et al., 1987). A medium inoculated with a larger number of spores will develop less aflatoxin than a medium inoculated with a smaller number of spores. The toxin production is apparently suppressed when the number of inoculated spores per unit volume of substrate exceeds a certain level (Karunaratne and Bullerman, 1990). The same effect was observed when barley was fumigated with phosphine or methyl bromide. Obviously, the reduction in the competing flora, either by irradiation or by fumigation, enhanced mycotoxin production (Chelak et al., 1991; Borsa et al., 1992).

In our study, the frequency of viable cells in Group 1 after irradiation was 36%. The irradiation decreased inoculum by three log cycles and increased the concentrations of fumonisins almost two-fold as compared with the control group. A reduction in the number of spores by approximately four log cycles, either by simple dilution or by irradiation, caused a two-fold increase in the toxin production by *A. parasiticus* (Sharma et al., 1980) and up to a 12-fold increase in the toxin production by *A. flavus* (Odamtten et al., 1987).

Table 3
Concentrations of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) in the grains of maize samples (mg/kg)

Sample no.	Control		Group 1		Group 2		Group 3	
	FB_1	FB_2	FB_1	FB_2	FB_1	FB_2	FB_1	FB_2
1	6.0	0.5	8.3	0.7	4.0	0.3	1.8	0.2
2	6.3	0.5	9.3	0.8	4.8	0.5	1.7	0.2
3	4.2	0.3	6.1	0.3	6.4	0.8	4.6	0.8
4	5.3	0.5	16.5	1.1	5.3	0.5	2.1	0.3
5	8.9	0.7	21.7	1.5	4.9	0.5	1.8	0.3
Mean (±SD)	6.1 (±1.74)	0.5 (±0.14)	12.3 (±6.50)	0.8 (±0.44)	5.08 (±0.87)	0.52 (±0.17)	2.4 (±1.23)	0.36 (±0.25)

Note: SD = Standard deviation.

Carefully selected processing methods can potentially result in lower fumonisin levels in maize-based products. It is important to keep in mind that their success will depend on many factors, including the moisture content of the product, the degree of contamination, distribution of the toxin in the product, and the presence of additives (Charmley and Prelusky, 1995; Bolger et al., 2001).

There are a few reports on the use of γ-radiation in controlling natural fumonisin in foods and on the effects of irradiation on the fumonisin molecular structure. Similar effects were found for aflatoxins (Rustom, 1997). More research is needed in order to better understand the interaction between γ-photons and fumonisins.

Removal of fumonisins from maize and maize-based products by means of chemical reactions is an object of many studies. Molecules of fumonisins are very stable, and their destruction is likely to be difficult. Ammoniation, initially used to detoxify aflatoxins, was tested as a means of fumonisin removal, but the results were not always satisfactory (Fandohan, 2003). Fumonisins also have relatively high thermo (Howard et al., 1998) and light (IARC, 1993) stability. Ordinary cooking does not reduce concentrations of this toxin substantially (Alberts et al., 1990; Scott, 1993). A significant decrease of fumonisin concentrations can be expected only in 15 min at temperatures above 218 °C (Castelo et al., 1998).

Table 4
Frequency (%) of viable fungal cells in unirradiated and irradiated maize samples as reported by the fluorescence DF-BE test

Sample no.	Control (0 kGy)	G1 (2 kGy)	G2 (5 kGy)	G3 (10 kGy)
1	72	36	5	0
2	83	34	6	0
3	75	38	7	0
Mean (±SD)	76.6 (±7.77)	36 (±1.41)	6 (±0.70)	0 (±0)

Note: SD = Standard deviation.

Aziz et al. (1997a, b) found that irradiation to 6 kGy eliminates fungi in wheat and wheat flour, but as much as 8 kGy was needed for complete degradation of mycotoxins. In this work, we observed that 10 kGy was not sufficient for a complete elimination of fumonisins. Visconti et al. (1996) investigated the effects of γ-radiation on naturally contaminated corn flour and found that 15 kGy sterilized the flour effectively and reduced its fumonisin content approximately by 20%.

3.2.2. Fluorescent viability test

Fluorescence methods based on FDA and EB can be used to determine the viability of fungal cells. The advantages of this test are quickness, high sensitivity, and the simplicity of the procedure (Calich et al., 1979). According to Lopes et al. (2002), both compounds, FDA and EB, have to be added simultaneously in order to obtain a strong contrast between living cells (green fluorescence) and dead cells (red fluorescence). Samples from the control group and Groups 1–3 were tested. In the control samples, 72–83% of cells were viable (produced green fluorescence). The viability decreased gradually with increasing radiation dose (Table 4). In Group 3, all cells were dead (emitted red fluorescent light). This confirmed the results of the plate counting, which did not detect any growth of colonies.

3.2.3. SEM

SEM offers higher resolution, amplification, depth of field, and versatility than optical microscopy (Goodhew and Humpreys, 1998). SEM studies by Torres et al. (2003)

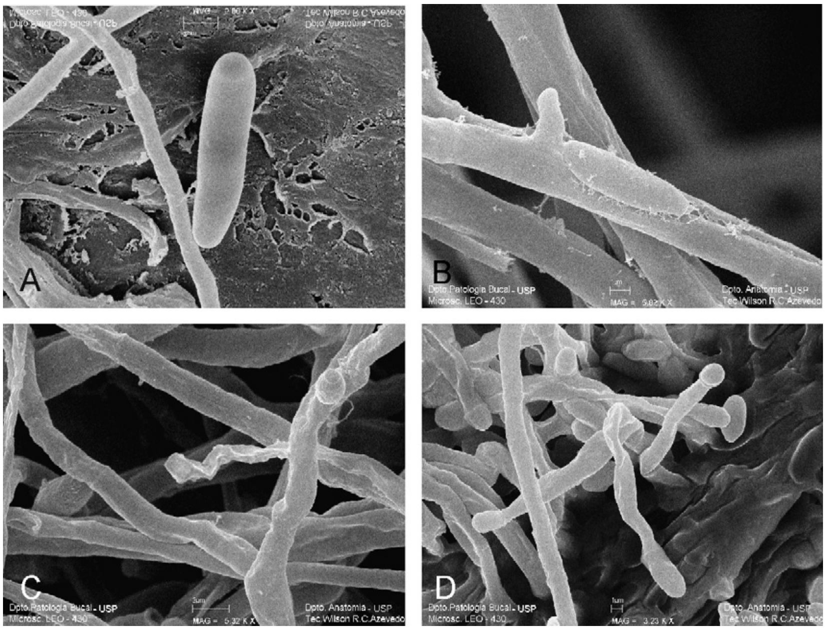

Fig. 1. Scanning electron microscopy images of unirradiated and irradiated maize samples: (A) unirradiated, (B) 2 kGy, (C) 5 kGy and (D) 10 kGy.

showed an effect of A_w and temperature on the growth of *A. ochraceus*, *Alternaria alternate*, and *F. verticillioides* in maize grains. Murillo et al. (1999) utilized SEM to observe the hyphae penetration of *F. moniliforme* in the grains of maize. Our results show that ionizing radiation causes modifications that increase with the radiation dose. The fungal structures in the samples of Groups 2 and 3 had torsions and ruptures in the hyphae and spores (Fig. 1). These changes can be attributed to the direct effect of radiation on the fungal cells.

4. Conclusions

γ-Radiation doses above 5 kGy effectively inhibit growth of *F. verticillioides* in maize grains, although a complete elimination of the fungal microflora requires 10 kGy. SEM revealed mycelium damage *F. verticillioides* by γ-radiation. The fluorescent viability test of spores showed that the Group 1 samples remained viable (36%) and the production of fumonisins increased after irradiation, even along with a reduction in fungal counting by three logarithmic cycles. This demonstrates that there is no direct correlation between the decrease in fungal counting (cfu/g) and the presence of mycotoxin. High doses (10 kGy) are necessary for eliminating mycotoxins or decreasing their concentrations to an acceptable level, as well as for killing mold. To reduce the risk of fungal growth in the food, it is necessary, first, to have a monitoring Program of Good Manufacturing Produce (GMP) and, second, to use γ-irrradiation. More studies of the effects of ionizing radiation on fumonisin molecules and the formed metabolites are necessary.

References

Alberts, J.F., Gelderblom, W.C.A., Thiel, P.G., Marasas, W.F.O., Van Schalkwyk, D.J., Behrend, Y., 1990. Effects of temperature and incubation period on production of fumonisin B_1 by *Fusarium moniliforme*. Appl. Environ. Microbiol. 56, 1729–1733.

Almeida, A.P., Fonseca, H., Fancelli, A.L., Direito, G.M., Ortega, E.M., Corrêa, B., 2002. Myclofora and fumonisin contamination in Brazilian corn from sowing to harvest. J. Agric. Food Chem. 50 (Suppl. 13), 3877–3882.

Aziz, N.H., Attia, E.S., Farag, S.A., 1997a. Effect of gamma-irradiation on the natural occurrence of *Fusarium* mycotoxins in wheat, flour and bread. Nahrung 41 (1), 34–37.

Aziz, N.H., El-Fouly, M.Z., Abu-Shady, M.R., Moussa, L.A.A., 1997b. Effect of gamma radiation on the survival of fungal and actinomycetal florae contaminating medicinal plants. Appl. Radiat. Isot. 48 (1), 71–76.

Bolger, M., Coker, R.D., Dinovi, M., Gaylor, D., Gelderblom, W., Olsen, M., Paster, N., Riley, R.T., Shephard, G., Speijers, G.J.A., 2001. Fumonisins. In: Safety Evaluation of Certain Mycotoxins in Food, WHO Food Additives Series 47, FAO Food and Nutrition Paper 74, Prepared by the 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO, Geneva, pp. 103–279. ISBN:9251046646.

Borsa, J., Chelak, W.S., Marquardt, R.R., Frohlich, A.A., 1992. Comparison of irradiation and chemical fumigation used in grain disinfestations on production of ochratoxin A by *Aspergillus alutaceus* in treated barley. J. Food Prot. 55, 990–994.

Bullerman, L.B., 1996. Occurence of *Fusarium* and fumonisins on food grains and in Foods. In: Jackson, L., De Vries, J.W., Bullerman, L.B. (Eds.), Fumonisins in Food. Plenum Press, New York, pp. 27–37.

Cahagnier, B., Melcion, D., Richard-Molard, D., 1995. Growth of *Fusarium moniliforme* and its biosynthesis of fumonisin B_1 on maize grain as a function of different water activities. Lett. Appl. Microbiol. 20, 247–251.

Calich, V.L., Purchio, A., Paula, C.R., 1979. A new fluorescent viability test for fungi cells. Mycopathologia 66 (3), 175–177.

Castelo, M.M., Sumner, S.S., Bullerman, L.B., 1998. Stability of fumonisins in thermally processed corn products. J. Food Prot. 61 (8), 030–1033.

Charmley, L.L., Prelusky, D.B., 1995. Decontamination of *Fusarium* mycotoxins. Appl. Environ. Microbiol. 1, 421–435.

Chelak, W.S., Borsa, J., Marquardt, R.R., Frohlich, A.A., 1991. Role of the competitive microbial flora in the radiation-induced enhancement of ochratoxin production by *Aspergillus alutaceus* var. alutaceus NRRL 3174. Appl. Environ. Microbiol. 57, 2492–2496.

Codex Alimentarius, 2003. Code of Practice for the prevention and reduction of mycotoxins contamination in cereals, including annexes on ochratoxin A, zearalenone, fumonisins and tricothecenes. CAC/RCP51, pp. 1–8.

Corrêa, B., Purchio, A., Paula, C.R., Gambale, W., Yasuda, M.A., 1990. Fluorescent method (fluorescein diacetate and ethidium bromide) to study the viability of *Cryptococcus neoformans* in liquor. Rev. Inst. Med. Trop. São Paulo 32 (1), 46–50.

Fandohan, P., Hell, K., Marasas, W.F.O., Wingfield, M.J., 2003. Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. Afr. J. Biotechnol. 2 (12), 570–579.

Geisen, R., 1998. PCR methods for the detection of mycotoxin-producing fungi. In: Bridge, P.D., et al. (Eds.), Applications of PCR in Mycology. CAB International, pp. 243–244.

Gelderblom, W.C.A., Jaskiewicz, K., Marasas, W.F.O., Thiel, P.G., 1991. Toxity and carcinogenity of the *Fusarium moniliforme* metabolite fumonisin B_1 in rats. Carcinogenesis 12 (4), 1247–1251.

Goodhew, P.J., Humpreys, F.J., 1998. Electron Microscopy and Analysis. Taylor & Francis, London.

Howard, P.C., Churchwell, M.I., Cough, L.H., Marques, M.M., Doerge, D.R., 1998. Formation of N-(carboxymethyl) fumonisin B_1, following the reaction of fumonisin B_1 with reducing sugars. J. Agric. Food Chem. 46, 3546–3557.

IARC, 1993. IARC monographs on the evaluation of carcinogenic risks to humans, vol. 56. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. International Agency for Research on Cancer, Lyon, France, 599.

Karunaratne, A., Bullerman, L.B., 1990. Interactive effects of spore load and temperature on aflatoxin production. J. Food Protect. 53, 227–229.

Kellerman, T.S., Marasas, W.F.O., Thiel, P.G., Gelderblom, W.C.A., Cawood, M., Coetzer, J.A.W., 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisins B_1. Onderstepoort J. Vet. Res. 57 (4), 269–275.

Lacey, J., Ramakrishna, N., Hamer, A., Magan, N., Marfleet, C., 1991. Grain fungi. In: Arora, D.K., et al. (Eds.), Handbook of Applied Micology: Foods and Feeds. Marcel Dekker, New York, pp. 12–77.

Ledoux, D.R., Brown, T.P., Weibking, T.S., Rottingaus, G.E., 1992. Fumonisin toxicity in broile chicks. J. Vet. Diagn. Invest. 4 (3), 330–333.

Leitão, M.F., 1998. Microbiologia dos alimentos. In: Rottmam, I., et al. (Eds.), Tratado de Microbiologia. Manole, São Paulo, pp. 1–81.

Lopes, M.A., Fischman, O., Gambale, W., Corrêa, B., 2002. Fluorescent method for studying the morphogenesis and viability of dermatophyte cells. Mycopathologia 156, 61–66.

Murillo, I., Cavallarin, L., San Segundo, B., 1999. Cytology of infection of maize seedlings by *Fusarium moniliforme* and immunolocalization of the pathogenesis-related PRms protein. Biochem. Cell Biol. 89 (9), 737–747.

Odamtten, G.T., Appiah, V., Langerak, D.I., 1987. Influence of inoculum size of *Aspergillus flavus* link on the production of aflatoxin B1 in maize medium before and after exposure to combination treatment of heat and gamma radiation. Int. J. Food Microbiol. 4, 119–127.

OMS, 1989. La iiradiación de los alimentos uma técnica para conservar y preservar la inocuidad de los alimentos. Genebra, Organización Mundial de la Salud, pp. 18–23.

Onyenekwe, P.C., Ogbadu, G.H., Hashimoto, S., 1997. The effect of gamma radiation on the micro flora and essential oil of Ashanti pepper (*Piper guineense*) berries. Postharvest Biol. Technol. 10, 161–167.

Orsi, R.B., Corrêa, B., Possi, C.R., Schammass, E.A., Nogueira, J.R., Dias, S.M.C., Malozzi, M.B.A., 2000. Myclofora and occurrence of fumonisins in freshly harvested and stored hybrid maize. J. Stored Prod. Res. 36, 75–87.

Osweiler, G.D., Ross, P.F., Wilson, T.M., Nelson, P.E., Witte, S.T., Carson, T.L., Rice, L.G., Nelson, H.A., 1992. Characterization of an epizootic of pulmonary edema in swine associated with fumonisins in corn screenings. J. Vet. Diagn. Invest. 4 (1), 53–59.

Pitt, J.I., Hocking, A.D., Glenn, D.R., 1983. An improved medium for the detection of *Aspergillus flavus* and *Aspergillus parasiticus*. J. Appl. Bacteriol. 54, 109–114.

Ramakrisna, N., Lacey, J., Smith, J.E., 1991. Effect of surface sterization, fumigation and gamma irradiation on the micro flora and germination of barley seeds. Int. J. Food Microbiol. 13, 47–54.

Riley, R.T., Voss, K.A., Norred, W.P., Sharma, R.P., Wang, E., Merril Jr., A.H., 1998. Fumonisins: mechanism of mycotoxicity. Revue Méd Vét 149 (6), 617–626.

Rustom, I.Y.S., 1997. Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods. Food Chem. 59 (Suppl. 1), 57–67.

Saleh, Y.G., Mayo, M.S., Ahearn, D.G., 1998. Notes: resistance of some common fungi to gamma irradiation. Appl. Environ. Microbiol. 54 (Suppl. 8), 2134–2135.

Scott, P.M., 1993. Fumonisins. Int. J. Food Microbiol. 18, 257–270.

Sharma, A., Behere, A.G., Padwal Desai, S.R., Nadkarni, G.B., 1980. Influence of inoculum size of *Aspergillus parasiticus* spores on aflatoxin production. Appl. Environ. Microbiol. 40, 989–993.

Sydenham, E.W., Shephard, G.S., Thiel, P.G., Stokenstrom, S., Snijman, P.W., 1996. Liquid chromatographic determination of fumonisins B_1, B_2 and B_3 in corn: AOAC–IUPAC collaborative study. J. AOAC Anal. Chem. Int. 79 (3), 688–696.

Torres, M.R., Ramos, A.J., Soler, J., Sanchis, V., Marín, S., 2003. SEM study of water activity and temperature effects on the initial growth of *Aspergillus ochraceus*, *Alternaria alternata* and *Fusarium verticillioides* on maize grain. Int. J. Food Microbiol. 81, 185–193.

Visconti, A., Solfrizzo, M., Doko, M.B., Boenke, A., Pascale, M., 1996. Stability of fumonisins at different storage periods and temperatures in gamma-irradiated maize. Food Addit. Contam. 13 (8), 929–938.

Winston, P.W., Bates, H.D., 1960. Saluted solution for the control of humidity in biological research. Ecology 41, 232–238.

World Health Organization (WHO), 1977. Wholesomeness of irradiated food. Technical Report Series, No. 604. Report of a Joint (FAO/IAEA) WHO Expert Committee.