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Effect of gamma irradiation doses and salting solutions (NaCl %) on the fumonisins (B_1 &B_2) of infected and row maize

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Abstract

The objectives of this investigation were to evaluate the effects and ability of gamma irradiation doses (4, 8 and 12 kGy) and salting solutions (7 %, 14% and 21% NaCl) to remove fumonisins (B_1&B_2) contaminated maize. Moreover, chemical and microbiological properties of maize affected by gamma irradiation doses were studied. The results indicated that: (i) there were not differences between non-irradiated and irradiated maize samples for its chemical composition; (ii)gamma rays had completely inactivated counts of total bacteria, molds and yeasts; (iii) a dose of 4, 8 and 12 kGy were sufficient for complete destruction of fumonisins infected maize; (iv) the fumonisins content in the row and deliberately infected maize decreased with the increasing concentration of NaCl; and (v) the combination of gamma irradiation and NaCl solutions inhibited of fumonisins. It can be concluded that gamma irradiation and NaCl solutions can be used for detoxification of fumonisin-contaminated maize and improving its microbial loud.

Keywords: Maize,Gamma irradiation, NaCl solution Mycotoxins, Fumonisins

1. Introduction

The occurrence, frequency and implication of mycotoxins entering the food and feed chain through cereal grain have gained global attention over the last decade (CAST, 2003). Natural carcinogens, such as mycotoxins, are present in foods as a result of contaminated raw materials or they may be produced during the processing and/or storage of foods. Fumonisins are fungal secondary metabolites produced by species of Fusarium, mainly Fusarium verticillioides and Fusarium proliferatum. There are several identified fumonisins, but fumonisin B1 (FB_1) and fumonisin B_2 (FB_2) are the most important and constitute up to 70% of the fumonisins found in naturally contaminated foods (CAST, 2003; Keller et al., 1997; Krska et al., 2007; Niderkorn et al., 2009; Romina et al., 2012). FB1 and FB2 are phytotoxic to corn (Lamprecht et al., 1994), cytotoxic to various mammalian cell lines (Abbas et al., 1993) and FB1 is carcinogenic to rat liver and kidney (IARC, 2002). This mycotoxin causes severe species-specific diseases in some livestock and laboratory animals, including kidney and liver cancer in rodents (Voss et al. 2001, 2002). A strong correlation between the consumption of maize highly contaminated with fumonisins and a high-incidence of human esophageal cancer has been detected in various regions of the world (Stockmann-Juvala and Savolainen, 2008). Consequently, fumonisin B_1 has been classified by the International Agency for Research on Cancer (IARC) as a Group 2B substance (potentially carcinogenic) (WHO-IARC, 2002). The FB1 inhibition capacity for ten natural phenolic compounds revealed thymol, carvacrol, and isoeugenol followed by eugenol to be the most active antifumonisin compounds (José et al., 2011). Reducing fumonisin contamination in corn will require greater understanding of how F. verticillioides infects and systemically colonizes corn tissues, and assessment of the impact that conidia may have on plantfungal interactions is essential for reduction of both plant disease and FB_1(Anthony, 2006). Methodology has not been devised to control fumonisin levels in maize and maize products during either pre- or post-harvest (Massimo et al., 2009). Most of strategies to prevent formation fumonisins, as well as to eliminate, inactivate or reduce their presence in food products have not been adopted due to high costs, loss of nutritional and sensory properties of the products, or practical difficulties involved in detoxification process (Firmin et al., 2011; Romina et al., 2012). Various methods of preservation have been applied to arrest growth of moulds in foods such as fumigation and heat treatment, but none of these methods offers complete control of toxigenic moulds. Ionizing radiation is one of the methods applied to decontaminate pathogenic microorganisms in different food commodities (Aziz & Moussa, 2004). Electron beam irradiation of Fusarium-infected barley reduced fungal infection at doses higher than 4 k. Gray (Kottapalli et al., 2003; Jean, 2007). High doses (>10 k Gray)

significantly decreased the germination capacity of barley grains. The use of gamma-irradiation can control the Fusarium mould growth as well as fumonisin B1 in wheat, maize and barley seeds. Further investigation is needed to clarify the interactions of gamma-irradiated fungal cells and food materials on mycotoxin production under different environmental conditions (Aziz, et al., 2007), who found that gamma 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. The objectives of this investigation were: I) to evaluate the capacity of a combination of gamma irradiation and salting solution to remove, as well as inactivate or reduce or eliminate FB_1 and FB_2 from deliberately infected maize and row infected maize; ii) to improve the microbiological safety of maize and iii) to determine the effect of gamma irradiation on chemical composition of maize.

2. Materials and methods

2.1. Materials

Maize seeds were collected from market in Banha, Kalubia Governorat, Egypt. These samples were kept at -4 ° C until analysis was carried out. Samples were analyzed in the Mycotoxin lab. In Regional Center for Food and Feed (RCFF), Egypt, for the presence of fumonisin B_1 and B_2.

2.2. Fungal strains

Pure strain of Fusarium moniliforme obtained from the Mycotoxin lab., National Research Center (NRC), Giza, Egypt.

2.3. Fumonisins standard

Mixtures of pure fumonisins standard (FB_1 and FB_2) with concentration 50µg/ml were purchased from Sigma-Aldrich Chemical Company, USA.

2.4. Salting solutions

Five hundred grams of contaminated or non- contaminated maize were added to one liter of water (0% NaCl), 7% NaCl, 14% NaCl or 21% NaCl solutions, stirred and allowed to stand for 30 min. After 30 min, the samples were separated, washed with excess water, blotted, and dried in a blow-air oven (at 50 º C) overnight while 500 grams of contaminated maize or non- contaminated was left without immersing in water or NaCl solution. The obtained samples were packed in polyethylene bags (each bag contains 100 grams) and the samples divided into two groups, the first group were used for irradiation process while the second group which contains five bags was left as control samples.

2.5. Irradiation process

Thirty bags (each bag contains 100 grams) from first group of contaminated and non-contaminated maize were gamma irradiated at 4,8, and 12 kGy doses using cobalt-60 gamma chamber (Dose rate 1.35814 kGy/h) in Cyclotron Project, Nuclear Research Center. Atomic Energy Authority, Inshas, Cairo, Egypt.

2.6. Preparation of Fumonisins contaminated maize (yellow corn):

Fumonisins were produced through fermentation of yellow corn with Fusarium moniliforme as described by Fadl-Allah et al. (1997). Fermentation was carried out in 2.5 L conical flasks containing 500g of washed yellow corn with distilled water and completely dried. 233 ml of water were added to each flask; then yellow corn was autoclaved at 121°C for 15min. Each flask was inoculated with 1 ml of Fusarium spore suspension contains 105 cfu and then was incubated at 20°C with moisture content of 40% for 5 weeks in the dark. Flasks were shaken once every day to prevent corn adhering and to distribute the inoculums. After incubation, the yellow corn was dried at room temperature, and then ground using an analytical mill and the fine powder was kept at 4°C for fumonisins analysis.

2.7. Sample preparation:

Each sample was ground in mill (1mm), homogenized and reduced up to 250g (sub-sample) and from this, 50 g portions were removed for analysis

2.8. Determination of Fumonisins concentration of maize (yellow corn) naturally contaminated and deliberately infected by Fusarium moniliforme

Fumonisins concentration was determined using HPLC technique (Agillent 1200 Series U.S.A with column C18, Kinetex 2.6u C18 100A 150x4.6mm, Phenomenex) as follows: The mobile phase consisted of methanol: 0.1M NaH2Po4 (77:23, v/v) at flow rat of 1ml/min. The excitation and emission wavelengths for all Fumonisins were 335nm, and 440 nm, respectively (Florences detector), according to (AOAC 2006) HPLC Chromatogram for the standard solution of fumonisin FB1 and FB2 were indicated in Fig.[1]

2.9. Chemical analysis

Moisture, oil, protein and ash contents were determined according to the stander methods of the (A.O.A.C. 1990). The total carbohydrates were calculated by difference according to (Egan et al., 1981) as the following: Total carbohydrates (%) = [100 – (moisture (%) + crude protein (%) + total lipids (%) + ash (%)].

2.10. Microbial Analysis:

Colony forming units for total bacterial counts were counted by plating on plate count agar medium and incubated at 30°C for 3-5 days (APHA, 1992).Total molds and yeasts were counted on oxytetracycline glucose yeast extract agar medium according to(Oxoid, 1998).

3. Results and Discussion

All measured values of chemical and microbiological properties are shown in Table [1], and as can be clearly seen, there were not significant differences between non-irradiated and irradiated maize samples for moisture, protein, oil, ash and fiber values. From the same table [1] showed that significant changes in microbiological properties and the viable counts of total bacteria, molds and yeasts reduced from 8.8× 10 3 and 5.4× 10 3 for non-irradiated row infected maize samples to 2.5× 102 and 6.9× 102 for irradiated row infected maize with 4 kGy gamma rays, respectively. And the samples irradiated at 8 and 12 kGy were completely inactivated. There has been increasing interest in the use of ionizing radiation for inhibiting the growth of microorganisms in different foods, livestock feed products and animal diets (Gharib & Aziz, 1995, Aziz, et al., 2007), they recorded that the total viable population of Aspergillus parasiticus decreased significantly by increasing gamma irradiation doses and irradiation at a dose 4.0 kGy reduced mould growth and there was no growth of the toxigenic moulds and mycotoxin in seeds using a dose of 5.0 kGy.

Table 1: Chemical and microbiological properties of non-irradiated and irradiated row infected maize seeds.

Chemical and microbiological Properties	Gamma irradiation doses			
	0 kGy	4 kGy	8 kGy	12 kGy
Chemical properties (%)				
Moisture [a]	8.80±0.935	8.70±0.804	8.60±0.299	8.90±0.772
Protein [a]	7.65±0.159	7.45±0.254	7.80±0.359	7.77±0.237
Oil [a]	3.77±0.363	3.96±0.230	3.84±0.081	3.72±0.230
Ash [a]	1.25±0.207	1.39±0.078	1.24±0.210	1.19±0.159
Fiber [a]	3.45±0.117	3.51±0.031	3.46±0.233	3.59±0.473
Microbiological properties				
Total bacterial count (CFU/g)[b*]	8.8× 10 ^{3}±2.16	2.5× 10 ^{2}±4.08	Nil	Nil
Total molds &yeasts (CFU/g)[b*]	5.4× 10 ^{3}±3.56	6.9× 10 ^{2}±4.32	Nil	Nil

a Values are mean of five measurements ± STDEV, with non significantly ($P < 0.05$).
b*Values are mean of four replicates ± STDEV, with significantly ($P < 0.05$) by increasing irradiation doses.

Table [2] shows that treatment of deliberately and row infected maize with gamma-irradiation doses at level of 4 kGy resulted in destruction of fumonisin B_1 and B_2. Also, from the same table it is clear that doses of 8 kGy and 12 kGy were sufficient for complete destruction of fumonisin $B_1$and B_2 in deliberately infected maize, while a dose of 4, 8 and 12 kGy were sufficient for complete destruction of fumonisin $B_1$and B_2 in row infected maize. The results of this study agree with the previous reports of (Aziz et al., 2007), these authors revealed that treatment of wheat, maize and barley seeds with gamma-irradiation at dose level of 3 kGy resulted in significant destruction of fumonisin B1 by 51.7%, 74.2% and 80% respectively, and they mentioned that a dose of 7 kGy was sufficient for complete destruction of fumonisin B1 in wheat and maize. So, from these reports and our present results, it appears that fungal strain, condition of storage and irradiation dose affect mould growth and

toxin production (AXatoxin B_1, ochratoxin A, zearalenone and fumonisin $B_1$and B_2) and further information regarding the ability of gamma radiation to destroy other mycotoxins in food commodities is needed.

Table 2: Effect of gamma-irradiation on the detoxification of fumonisin levels (B_1 and B_2) i in deliberately and row infected maize.

Fumomisin (µg/g)	Gamma irradiation doses			
	0 kGy	4 kGy	8 kGy	12 kGy
Deliberately infected maize				
FB_1	5.280	0.321	ND	ND
FB_2	2.020	ND	ND	ND
$FB_1 + FB_2$	7.300	0.321	ND	ND
Row infected maize				
FB_1	1.100	ND	ND	ND
FB_2	0.321	ND	ND	ND
$FB_1 + FB_2$	1.421	ND	ND	ND

ND: not detected

From data in table [3] It was observed that the fumonisin B_1 and B_2 content in the deliberately and row infected maize decreased with the increasing concentration of NaCl. It decreased from 5.944; 0.321 µg/g for deliberately and row infected maize samples treated with water (0% NaCl solution) to (4.900; 0.220), (4.300; 0.190) and (4.200; 0.180) µg/g for deliberately and row infected maize samples treated with 7, 14 and 21% NaCl solution, respectively.

Table 3: Effect of salting solutions (NaCl %) on the detoxification of fumonisin levels (B_1 and B_2) in deliberately and row infected maize.

Fumomisin (µg/g)	Salting solutions (Na Cl%)			
	Water (0%)	7%	14%	21%
Deliberately infected maize				
FB_1	4.421	3.927	3.570	3.448
FB_2	1.523	0.973	0.730	0.642
$FB_1 + FB_2$	5.944	4.900	4.300	4.200
Row infected maize				
FB_1	0.321	0.220	0.190	0.180
FB_2	ND	ND	ND	ND
$FB_1 + FB_2$	0.321	0.220	0.190	0.180

ND: not detected

Table [4 & 5] showed that the concentrations of fumonisins $B_1$and B_2 in our samples also changed as a result of combination of gamma irradiation and concentration of NaCl % solutions. It decreased with increasing of NaCl % solutions and the lower concentration were noticed in deliberately and row infected maize samples treated by 21% NaCl solution and irradiated with 4 kGy gamma rays. From the same table 4 it could be noticed that the combination of gamma irradiation and concentration of NaCl solutions had effectively inhibited and detoxification of fumonisin B_1 and B_2, this results duo to gamma irradiation has been tested to reduce fungal spore contamination of seeds, food or feeds or to degrade mycotoxins already produced (jean, 2007). Gamma irradiation at a dose 5 kGy gamma ray inactivated the growth of Fusarium sp. and mycotoxin formation in seeds (Aziz and Moussa, 2004. 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.

Table 4: Effect of combination treatments of gamma irradiation and salting solutions (NaCl %) on the detoxification of fumonisin levels (B_1 and B_2) in deliberately infected maize.

Salting solutions (NaCl%)	Fumomisin (µg/g)	Gamma irradiation doses		
		4 kGy	8 kGy	12 kGy
Water (0%)	FB_1	0.295	ND	ND
	FB_2	ND	ND	ND
	$FB_1 + FB_2$	0.295	ND	ND
(7%)	FB_1	0.244	ND	ND
	FB_2	ND	ND	ND
	$FB_1 + FB_2$	0.244	ND	ND
(14%)	FB_1	0.233	ND	ND
	FB_2	ND	ND	ND
	$FB_1 + FB_2$	0.233	ND	ND
(21%)	FB_1	0.201	ND	ND
	FB_2	ND	ND	ND
	$FB_1 + FB_2$	0.201	ND	ND

ND: not detected

Table 5: Effect of combination treatment of gamma irradiation and salting solutions (NaCL %) on the detoxification of fumonisin levels (B_1 and B_2) in row infected maize.

Salting solutions (NaCl%)	Fumomisin (µg/g)	Gamma irradiation doses		
		4 kGy	8 kGy	12 kGy
Water (0%)	FB_1	0.150	ND	ND
	FB_2	ND	ND	ND
	$FB_1 + FB_2$	0.150	ND	ND
(7%)	FB_1	0.149	ND	ND
	FB_2	ND	ND	ND
	$FB_1 + FB_2$	0.149	ND	ND
(14%)	FB_1	0.148	ND	ND
	FB_2	ND	ND	ND
	$FB_1 + FB_2$	0.148	ND	ND
(21%)	FB_1	0.146	ND	ND
	FB_2	ND	ND	ND
	$FB_1 + FB_2$	0.146	ND	ND

ND: not detected

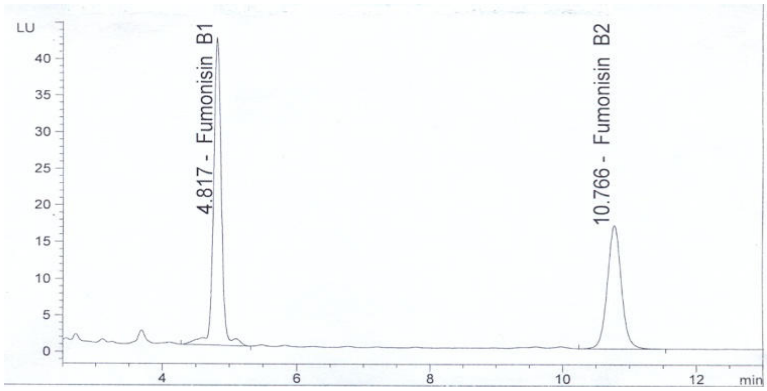

Fig. 1. HPLC Chromatogram standard solution at concentration 5µg/ml FB_1 and FB_2

Conclusions

This work demonstrates that gamma radiation doses above 4 kGy effectively inhibit growth of total bacteria, molds and yeasts in maize grains, a complete elimination of the fungal microflora requires 8 and 12 kGy and these doses are necessary for eliminating mycotoxins or decreasing their concentrations to an acceptable level, as well as for killing mold. Moreover, to reduce the risk of fungal growth and natural fumonisins (B_1 &B_2) toxins in the maize grains, it is necessary to use combination of gamma irradiation and sodium chloride solution.

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