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Food Control 13 (2002) 281–288

FOOD CONTROL

www.elsevier.com/locate/foodcont

Influence of gamma-radiation on mycotoxin producing moulds and mycotoxins in fruits

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Received 18 July 2001; received in revised form 2 February 2002; accepted 4 February 2002

Abstract

One hundred random fruit samples were collected and analyzed for mycotoxins and the effect of gamma-irradiation on the production of mycotoxins in fruits was studied. Analysis of fruits revealed the occurrence of penicillic acid, patulin, cyclopiazonic acid (CPA), citrinin, ochratoxin A and aflatoxin B_1. Of the 100 samples examined, 60 were positive for one or more mycotoxin. Irradiation of fruits at dose of 1.5 and 3.5 kGy decreased significantly the total fungal counts compared with unirradiated controls. After 28 days of storage at refrigeration temperature, the unirradiated fruits were contaminated with high concentrations of mycotoxins as compared with irradiated 3.5 kGy samples. Mycotoxins production in fruits decreased with increasing irradiation dose and were not detected at 5.0 kGy.

Keywords: Aflatoxin; Cyclopiazonic acid; Ochratoxin A; Patulin; Pencillic acid; *Aspergillus flavus*; *Pencillium* sp. *Aspergillus* sp.; Moulds; Fruit contamination; Food spoilage; Toxinogenic fungi; Biodeterioration; Gamma-irradiation; Refrigeration; Storage

1. Introduction

Moulds of the genera *Aspergillus* and *Penicillium* occur in different fruits (Hall & Scott, 1977). Fruits become increasingly susceptible to fungal invasion during ripening as the pH of the tissue increases, skin layers soften, soluble carbohydrates build up and defence barriers weaken (Pitt & Hocking, 1985). Several mycotoxins are known to be produced by *Aspergillus* and *Penicillium* species and many different isolates have been described as toxinogenic (Trucksess, Mislivec, Young, Bruce, & Page, 1987). Mycotoxins have been found as natural contaminants in different food and feed commodities in Egypt (Aziz & Youssef, 1991; Aziz, Refai, & Abd El-Aal, 1990). The contamination of food and feed by these mycotoxins can lead to health problems in human and animals and can result in economic losses (Rodrick, 1976). The purpose of this study was to: (1) isolate and identify fungal species and their toxigenic capacity, (2) to estimate the natural occurrence of mycotoxins in fruits and (3) to study the effect of gamma-irradiation on mycotoxin production in fruits during storage under refrigeration.

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2. Materials and methods

2.1. Fruit collection

A total of 100 fruit samples: 10 each of strawberry, apricot, plum, peach, grapes, date, fig, apple, pear and mulberry were purchased from different grocery stores in Cairo and Giza areas, Egypt. No more than 10 products were purchased at any one time. Samples were purchased from the local market; three lots of 1 kg each were analyzed. These were not premium grade fruits but showed characteristic brown discoloration on the outside. In same samples, rotting had just started with a patch of slight green mould growth on some samples. On receipt, samples were stored at 4 °C until mould had been isolated and mycotoxin analysis was carried out.

2.2. Fungal isolation and identification

Samples of about 250 g each were sliced and thoroughly mixed, then 50 g was used for preparing dilutions for fungal counts and isolation. The samples (50 g) were homogenized for 3 min with 450 ml sterile distilled water. For fungal counts and identification, 1 ml of the dilutions was plated using Czapek–Dox agar, potato

0956-7135/02/$ - see front matter
PII: S0956-7135(02)00028-2

dextrose agar and malt agar media. After an incubation for 7 days at 23 °C, fungal colonies were counted. Fungi were subcultured and later identified according to Pitt (1985), and Pitt and Hocking (1985). Fungal isolates were sent to the Central Bureau voor Schimmel Cultures, Baarn, The Netherlands and to the Mycotoxicology Department, Animal Health Research Institute, Giza, Egypt to confirm identification.

2.3. Production of mycotoxins by isolated moulds

Pure isolates of *Aspergillus* and *Penicillium* were grown on Czapek agar plates. After 7 days the isolates were transferred to freshly prepared synthetic liquid medium (Dorner, Cole, & Diener, 1984). The medium contained 5 g of Difco mycological peptone, 20 g of yeast extract, 40 g of sucrose and 1 l of deionized water (pH 5.5). Portions of the medium (100 ml), were poured into 250 ml Erlenmeyer flasks, sealed with cotton plugs and autoclaved for 20 min at 121 °C. After cooling, the flasks were inoculated with 10^7 fungal spores and were kept stationary in the dark for 14 days at 28 °C. *Aspergillus flavus* NRRL 3251 known to produce cyclopiazonic acid (CPA) and aflatoxin B_1, *A. flavus* NRRL 6388 known to produce CPA but not aflatoxin, *Penicillium urticae* NRRL 994, a known patulin-producing organism and *P. urticae* KXL 301, known to produce CPA only (F.M. EL-Far, Mycology Department, Animal Health Research Institute, Dokki, Giza, personal communication, 1997) were also cultured for production of mycotoxins.

2.4. Extraction and analysis of mycotoxins

At the end of the incubation period, the entire fermentation mixture in each flask was extracted with 100 ml chloroform and shaken at 200 rpm on a rotary shaker for 24 h. The chloroform layer was decanted and retained. Chloroform extraction was repeated and the two chloroform extracts were combined, filtered through anhydrous sodium sulfate, and concentrated to a small volume by evaporation under vacuum on a boiling water bath. Chloroform extracts were then cleaned up by column chromatography (A.O.A.C., 1984).

2.5. Chromatographic analysis of chloroform extracts

Thin layer chromatography (TLC) was routinely used for quantitative and qualitative estimations of aflatoxin, ochratoxin A, CPA, patulin, citrinin and penicillic acid.

2.6. Assay of aflatoxins

Aflatoxins were separated by TLC on Kiesselgel 60 using 20×20 cm^2 plates (Merck, GFR) with 0.25 mm thickness. Four solvent systems were used, chloroform–acetone $(85 + 15)$, chloroform–acetone $(90 + 10)$, chloroform–methanol $(96 + 4)$ and chloroform–ethyl acetate–water $(85.5 + 12 + 2.5)$. All extracts were first eluted by ethyl ether (anhydrous)–methanol–water $(96 + 3 + 1)$ (A.O.A.C., 1984). Identification and estimation of aflatoxin quantity were performed visually under long wave UV light (365 nm) by comparison with reference standards. Aflatoxin B_1 was confirmed by reaction with trifluoroacetic acid and by the change to yellow fluorescence with 25% sulfuric acid in accordance with conditions approved by the A.O.A.C. (1984).

2.7. Determination of cyclopiazonic acid

The TLC plates were dipped in 2% alcoholic oxalic acid solution and dried at 80 °C for 1 h. The chloroform residue was redissolved in 200 μl chloroform and 2 and 5 μl aliquots of the chloroform solution were applied on the plate along with 2, 4 and 10 μl CPA standards (100 ng/μl chloroform). The plate was developed with benzene–acetic acid–methanol $(90 + 5 + 7)$ and dried at room temperature. CPA was visualized with spray reagent 1% *p*-dimethyaminobenzaldehyde in 75 ml ethanol and 25 ml HCl. CPA was detected as a characteristic bluish purple spot both in visual and UV light with Rf 0.60 and confirmed by rechromatography with standard toxin. The identify of CPA in the positive samples was further confirmed by mass spectrometry according to the method of Trucksess et al. (1987).

2.8. Determination of ochratoxin A, citrinin, patulin and penicillic acid

Each sample residue was dissolved in 0.5 ml chloroform and 25 μl was spotted on a TLC plate, 10 μl of standard of each mycotoxins (100 μg/ml in chloroform), were also spotted on the TLC plate. The TLC plates were allowed to run in a solvent of toluene–ethyl acetate–90% formic acid $(60 + 30 + 10)$. Dried plates were examined under long wave UV light; spots were compared with standards before and after exposure to ammonia vapor for 10 min. Further confirmation for positive samples was carried out (Golinski & Grabarkiewicz-Szczesna, 1984).

2.9. Analysis of fruit samples for mycotoxins

Samples of about 200 g were blended for 3 min at high speed with 800 ml AR chloroform. Each homogenate was filtered through a fluted Whatman No. 1 filter paper and 50 ml filtrate was collected, the filtrate was passed through a cleanup column of anhydrous sodium sulfate and washed with 100 ml chloroform. The

combined chloroform extracts were evaporated from the sample and the final residue was transferred to a glass vial using small volumes of AR chloroform. Mycotoxins were quantified by direct comparison of the spots of sample extracts with appropriate dilutions of standard mycotoxin solutions, as described before. Mycotoxin identity was further confirmed by high-pressure liquid chromatography (HPLC). The HPLC (Waters Associates, Milford, MA) was equipped with dual Model M6000 pumps, a model 440 UV detector (340 nm emission filter), and a U 6 K septumless injector. Each of the four standard solutions ranging in concentrations from 0.1 to 0.001 mg/ml was injected 10 times into the chromatograph. The peak height ratio for the samples agreed within 10% of that obtained for the standard mycotoxin (Roberts & Patterson, 1983).

2.10. Irradiation

Fruit samples (500 g) were sealed in polyethylene plastic bags and irradiated at room temperature (25 °C) under ambient atmosphere in the National Center for Radiation Research and Technology, Cairo, Egypt, Co^{60} gamma facility "Gamma Cell model 220 apparatus". The radiation doses selected for evaluation were 1.5, 3.5 and 5.0 kGy delivered at dose rate of 1.8 kGy/h. The numbers of fungal colony-forming units were determined by serially diluting the preparations and plating duplicate samples onto potato dextrose agar. The plates were incubated for 4 days at 28 °C, the colonies were counted and the data were expressed as the number of colony-forming units per gram. The unirradiated and irradiated fruit samples were stored for 25 days at refrigeration (below 10 °C). After 14 and 28 days, the numbers of fungal colony forming units were determined for the unirradiated and irradiated fruit samples. At the end of the storage period (28 days) all unirradiated and irradiated (3.5 kGy) samples were analyzed for the occurrence of mycotoxins as determined previously using HPLC.

2.11. Statistical analysis

Least significant difference (LSD) was used for comparing treatment means (Snedecor & Cochran, 1980).

3. Results and discussion

3.1. Isolation and identification-toxigenic capacity

Table 1 shows that samples such as strawberry, peach, grape, fig, apple, pear and mulberry had the highest total fungal, *Penicillium* sp. and *Aspergillus* sp. count whereas, apricot, plum and date had the lowest fungal counts. Domsch, Games, and Andreson (1980) reported that contamination of food commodities with fungal species was as a result of natural extraneous contamination by dust followed by holding under humid conditions. The data obtained in Table 1 indicated that almost all fruit samples examined in this study were contaminated with moulds. *Penicillium* sp. (68–52%) were the most abundant fungi followed by *Asperigillus* sp. (23–33%). Mycological analysis of 100 fruit samples revealed six genera of contaminating fungi, *Aspergillus*, *Penicillium*, *Botrytis*, *Rhizopus*, *Rhizoctonia* and *Alternaria* (Table 2). With all fruit samples examined, the flora consisted of soil fungi, completely in agreement with other investigations (Aziz, 1987; Shotwell, Goulden, Hesseltine, Dickens, & Kwolek, 1980), and storage fungi such as *Aspergillus* and *Penicillium* sp. (Pitt & Hocking, 1985). Table 2 indicates that *Alternaria alternata*, *Botrytis cinerea*, *Rhizopus nigricans* and *Rhizoctonia solani*, *Aspergillus* and *Penicillium* sp. were found in fruit samples. The greater number of species belonged to the genus *Penicillium*. Ten species were recovered, the most common were: *P. chrysogenum* 77%, *Penicillium aurantiogriseum* (formerly *cyclopium)* 75%, *P. griseofulvum* (formerly *urticae*) 81% and *P. verrucosum* 67%. Six species of *Aspergillus* were isolated, the most common were *Aspergillus niger* 82%, *A. flavus* 48% and

Table 1
Total fungal, *Penicillium* and *Aspergillus* counts in fresh fruits

Samples	Total count per gram	*Penicillium* sp.		*Aspergillus* sp.		Other genera	
		Total count per gram	%	Total count per gram	%	Total count per gram	%
Strawberry	6.3×10^5	3.8×10^5	60.8	1.8×10^5	28.80	6.5×10^4	10.40
Apricot	4.1×10^4	2.3×10^4	56.10	1.2×10^4	29.27	6.0×10^3	14.63
Plum	8.5×10^4	5.6×10^4	66.12	2.1×10^4	24.44	8.0×10^3	9.45
Peach	3.3×10^5	1.8×10^5	54.55	9.0×10^4	27.27	6.0×10^4	18.18
Grape	2.8×10^5	1.7×10^5	58.93	8.0×10^4	28.57	3.5×10^4	12.50
Date	5.2×10^4	2.7×10^4	51.94	1.4×10^4	27.33	1.1×10^3	20.74
Fig	6.0×10^5	4.1×10^5	68.33	1.4×10^5	22.50	5.5×10^4	9.17
Apple	7.3×10^5	4.7×10^5	64.55	1.7×10^5	22.67	9.2×10^4	12.69
Pear	4.0×10^5	2.4×10^5	58.75	1.3×10^5	32.50	3.5×10^4	8.75
Mulberry	3.3×10^5	2.0×10^5	61.66	9.0×10^4	27.61	3.5×10^4	10.74

Mean of 10 samples of each fruit.

Table 2
Moulds isolated from fruit samples[a]

Species of fungi	Frequency of occurrence									
	Strawberry	Apricot	Plum	Peach	Grape	Date	Fig	Apple	Pear	Mulberry
A. alternata	30	30	10	20	30	10	10	20	30	40
A. candidus	30	0	0	60	30	0	0	20	40	0
A. flavus	80	90	70	100	80	60	80	80	100	100
A. niger	100	80	100	60	80	60	100	90	80	70
A. ochraceus	60	60	80	60	70	80	60	70	60	80
A. sclerotiorum	10	20	40	0	0	30	0	0	20	0
A. terreus	0	30	20	0	10	0	0	0	0	0
B. cinerea	80	60	70	80	60	70	80	60	100	100
Penicillium brevicompactum	40	0	60	50	0	30	20	10	40	40
P. chrysogenum	80	60	80	100	80	60	80	90	80	60
P. citrinum	0	10	0	0	20	0	0	30	10	0
P. aurantiogriseum	80	70	80	100	60	80	80	70	70	60
P. commune	30	0	40	0	0	30	10	0	0	0
P. expansum	0	0	90	80	40	10	80	90	60	60
P. oxalicum	40	30	0	50	10	30	0	40	0	10
P. griseofulvum	80	10	60	90	80	70	60	100	100	70
P. islandicum	0	10	30	0	30	50	0	60	40	50
P. verrucosum	70	30	10	80	80	30	50	60	70	100
R. nigricans	60	40	80	80	30	40	50	10	10	20
R. solani	90	30	40	40	30	40	10	20	30	30

[a] Number of samples detected/number tested (%).

Aspergillus ochraceus 68%. Hall and Scott (1977) found that *Penicillium* sp. and *B. cinerea* are most common destructive fungal spoilage in apples and pears. Gathercole, Wachtel, Magarey, and Stevens (1987), and Sharma, Kaul, and Jindal (1990) revealed that *A. flavus*, *A. niger*, *A. alternata*, *B. cinerea*, *Cladosporium herbarum*, *Penicillium cyclopium*, *P. viridicatum*, *P. urticae*, *Trichothecium roseum*, *Rhizopus stolonifer* and *Verticillium* sp. were the most prevalent in peach, plums, apricots, nectarines, cherries and table grapes. These observations are in agreement with our present data that nearly all fruit samples studied were contaminated with almost all the above genera and species.

Table 3 lists results for mycotoxins with 31 *Aspergillus* and 65 *Penicillium* isolates screened. In this study all isolates of *A. flavus* were aflatoxin B_1 (nine isolates) and CPA (four isolates) positive. The control isolate *A. flavus* (NRRL 3251) produced CPA and aflatoxin, while *A. flavus* (NRRL 6388) produced CPA. Also, Table 3 reveals that all isolates of *A. ochraceus* (eight isolates) were ochratoxin A-positive and addition, *Aspergillus candidus* (eight isolates), and *A. terreus* (two isolates) were aflatoxin, CPA and ochratoxin A-negative. In a previous study, Trucksess et al. (1987) found that one isolate of *A. tamarii* and 19 of 31 isolates of *A. flavus* were CPA-positive and in only 6 isolates of *A. flavus* produced

Table 3
Mycotoxins produced by *Aspergillus* sp. and *Penicillium* sp. in liquid medium at 28 °C

Fungal sp.	No. of isolates examined	No. producing isolates (conc. μg/ml)						Total of isolates
		Aflatoxin B1 (AFB1)	Cyclopiazonic acid (CPA)	Ochratoxin A	Penicillic acid	Patulin	Citrinin	
A. candidus	8	ND	ND	ND	ND	ND	ND	0
A. flavus[*]	13	9 (800–3000)	4 (65–150)	ND	ND	ND	ND	13
A. terreus	2	ND	ND	ND	ND	ND	ND	0
A. ochraceus	8	ND	ND	8 (175–400)	ND	ND	ND	8
P. aurantiogriseum	42	ND	ND	ND	23 (200–420)	ND	ND	23
P. griseofulvum	16	ND	4 (25–75)	ND	ND	12 (330–550)	ND	16
P. expansum	2	ND	ND	ND	ND	2 (225, 300)	ND	2
P. verrucosum	1	ND	ND	1 (870)	ND	ND	ND	1
P. citrinum	4	ND	ND	ND	ND	ND	4 (170–250)	4
Total	96	9 (9.38%)	8 (8.33%)	9 (9.38%)	23 (23.96%)	14 (14.58%)	4 (4.17%)	67 (69.79%)

ND: not detected.
LQ (μg/ml): aflatoxin $B_1 = 50$, CPA = 50, ochratoxin A = 100, penicillic acid = 100, patulin = 50, citrinin = 50.
LD (μg/ml): aflatoxin $B_1 = 100$, CPA = 30, ochratoxin, A = 100, penicillic acid = 50, patulin = 100, citrinin = 200.
[*] None of these produced both AFB1 and CPA.

Table 4
Detection of mycotoxins in different fruit samples before storage

Source	No. of samples examined	No. of positive samples	No. of positive samples (conc. μg/kg)					
			Aflatoxin B_1	Cyclopiazonic acid	Ochratoxin A	Penicillic acid	Patulin	Citrinin
Strawberry	10	8	–	2 (125)	–	3 (160–180)	3 (170–200)	–
Apricot	10	5	–	–	–	1 (135)	4 (150–170)	–
Plum	10	9	2 (150)	–	–	3 (125–130)	4 (180–200)	–
Peach	10	10	–	–	3 (260–300)	6 (100–170)	1 (150)	–
Grape	10	4	–	2 (120)	–	–	–	2 (70)
Date	10	1	1 (185)	–	–	–	–	–
Fig	10	1	–	–	–	–	–	1 (60)
Apple	10	10	–	–	–	6 (140–180)	4 (200)	–
Pear	10	7	–	2 (150)	–	1 (135)	3 (150–200)	1 (50)
Mulberry	10	5	–	–	2 (280–300)	1 (100)	2 (150–170)	–
Total	100	60	3	6	5	21	21	4

(–) No detection of mycotoxins.
(LD) Detection limits (μg/kg): aflatoxin B_1: 0.1–0.3, ochratxin A = 5–10, CPA = 10–20, penicillic acid = 10–40, patalin = 20–100, citrinin = 40–100.
(LQ) Quantification limits (μg/kg): 5 μg/kg for aflatoxin B_1, CPA and ochratoxin A and 20 μg/kg for penicillic acid, palulin and citrinin.

aflatoxin and CPA. *A. flavus* and *A. parasitcus* were observed as the main fungal species producing aflatoxins in food and feed products (Aziz, 1987; Aziz et al., 1990). Dorner et al. (1984) and Trucksess et al. (1987) reported that *A. flavus*, *A. versicolor*, *A. oryzae* and *A. tamarii* were CPA producers. In addition from Table 3 it is clear that out of 42 isolates of *P. aurantiogriseum* only 23 isolates were penicillic acid-positive. In addition all 16 isolates of *P. griseofulvum* produced patulin (12 isolates) and CPA (four isolates). The control isolate *P. urticae* (NRRL 994) produced patulin while *P. urticae* (KXL 301) produced CPA. Also, from this table the two isolates of *P. expansum* were patulin producers, the one isolate of *P. verrucosum* was ochratoxin A-positive and all of the four isolates of *P. citrinum* were citrinin-positive. Penicillic acid is a well known mycotoxin produced in large amounts by strains of *Penicillium* sp., among these are *P. cyclopium*, *P. puberulum*, *P. martensii* and *P. palitans* (Pitt & Hocking, 1985). Leistner and Pitt (1977) revealed that CPA was produced by *P. cyclopium,* ochratoxin by *P. verrucosum*, and citrinin, patulin and penicillic acid by many penicillia. The mycotoxin ochratoxin A was originally discovered during a pure culture study of *A. ochraceus* (Aziz, 1987; Refai, Aziz, El-Far, & Hassan, 1996). In the present studies, it is clear that most isolates of *Aspergillus* and *Penicillium* sp. produce mycotoxins in relatively high amounts. Their high incidence rate in fruits suggested that fruit samples must be investigated for the natural presence of these mycotoxins.

3.2. Mycotoxin analysis

One hundred fruit samples have been analyzed in this survey of mycotoxin contamination. Table 4 summarizes the total number and types of samples tested. Of the 100 samples of fruits, only 60 samples were contaminated with mycotoxins. Patulin and penicillic acid were detected in 21 of 60 samples of fruits at levels from 150 to 200 μg/kg and from 100 to 180 μg/kg, respectively. Strawberry, apricot, plum, peach, apple and mulberry were the most important fruits contaminated with both patulin and penicillic acid. Aflatoxin B_1 was detected in two samples of plum at the level 150 μg/kg and in one sample of date at level 185 μg/kg. On the other hand, CPA was detected in two samples each of strawberry, grape and pear at concentrations of 125, 120 and 180 μg/kg, respectively. In addition from this table, it is clear that peach (three samples) and mulberry (two samples) were contaminated with ochratoxin A at levels of 260 and 300 μg/kg, respectively, and grape (two samples), fig (one sample) and apple (one sample) were contaminated with citrinin at levels of 70, 60 and 50 μg/kg, respectively. Generally, in this study all mouldy fruit samples examined were contaminated naturally with one or more mycotoxins. Neelakantan, Balasubramanian, Jasmine, and Balasaraswathi (1983) reported the analysis of grapes, bananas, apples, tomatoes, papaya and oranges and they did not detect penicillic acid in any samples except in one sample of apple (60 μg/kg). Numerous reports have been published on the appearance of patulin in apple products at levels ranging from 75 to 200 μg/kg (Kubacki, 1986). It is also been reported that citrinin occurred naturally in rotted apples containing *P. expansum* (Hall & Scott, 1977).

3.3. Effect of gamma-irradiation on fungal population and mycotoxins in fruits

In this study the effect of gamma irradiation on the initial fungal population of fruit samples stored at refrigeration (below 10 °C) for 28 days is shown in Table 5. It is clear that the initial viable population of fungi of

Table 5
Influence of gamma-irradiation on the total fungal count in local fruit samples stored at refrigeration for various storage periods

Samples	Number of colony forming units per gram								
	0 days			14 days			28 days		
	0	1.5 kGy	3.5 kGy	0	1.5 kGy	3.5 kGy	0	1.5 kGy	3.5 kGy
Strawberry	3.7×10^5	1.7×10^2	3.0×10^1	6.1×10^5	2.2×10^2	4.0×10^1	8.8×10^6	2.8×10^2	1.0×10^1
Apricot	4.8×10^4	2.2×10^2	3.0×10^1	7.0×10^4	2.6×10^2	6.0×10^1	6.5×10^5	3.3×10^2	1.0×10^1
Plum	8.6×10^4	4.6×10^2	1.7×10^2	8.2×10^4	5.1×10^2	1.8×10^2	1.5×10^5	6.0×10^2	1.2×10^1
Peach	3.0×10^5	3.1×10^3	2.6×10^2	4.6×10^6	3.6×10^3	1.5×10^2	8.7×10^6	5.4×10^2	1.7×10^1
Grape	2.8×10^5	2.5×10^3	1.2×10^2	5.8×10^6	3.0×10^3	1.4×10^2	7.6×10^6	5.0×10^2	1.0×10^1
Date	4.8×10^4	5.0×10^2	3.0×10^1	4.7×10^4	8.0×10^2	5.0×10^1	6.6×10^6	8.0×10^2	2.0×10^1
Fig	5.7×10^5	1.7×10^3	2.0×10^2	1.3×10^6	3.1×10^3	1.6×10^2	5.8×10^6	4.0×10^2	2.0×10^1
Apple	6.8×10^5	2.8×10^3	1.7×10^2	5.9×10^5	3.5×10^3	1.8×10^2	4.9×10^6	5.0×10^2	1.4×10^1
Pear	6.0×10^5	1.4×10^2	1.4×10^1	6.3×10^5	1.9×10^3	1.2×10^1	3.7×10^6	2.5×10^2	1.3×10^1
Mulberry	3.1×10^5	3.1×10^3	1.4×10^2	1.3×10^6	4.5×10^3	1.8×10^2	6.4×10^6	6.8×10^2	1.3×10^1

(1) The total fungal counts in unirradiated fruit samples increased significantly ($P < 0.05$) by increasing the storage periods. (2) The total fungal counts in fruit samples decreased significantly ($P < 0.05$) at irradiation dose levels 1.5 and 3.5 kGy. (3) There was no significant increase in the total fungal counts in irradiated fruit samples by increasing the storage periods.

fruit samples ranged from 4.8×10^4 to 6.8×10^5 per gram. When fruit samples were irradiated at dose level 1.5 and 3.5 kGy, the total fungal counts were reduced (1.4×10^2–2.5×10^3 and 1.4×10^1–2.5×10^2 per gram, respectively). After 14 days of storage at refrigeration, the total fungal counts ranged from 2.2×10^2 to 4.5×10^3 per gram and from 1.2×10^1 to 1.8×10^2 per gram after exposure of fruits to 1.5 and 3.5 kGy radiation dose, respectively, whereas the fungal counts of the unirradiated samples increased to 4.7×10^4–5.8×10^6 per gram of fruit. Our results (Table 5) also indicate that after 28 days of storage at refrigeration, the total fungal counts of 1.5 and 3.5 kGy irradiated fruit samples had an average of 4.88×10^2 and 1.39×10^1 per gram, respectively, whereas the fungal counts for the unirradiated fruit samples increased up to about 6.05×10^6 per gram. Our results indicated that the fungal flora in the different fruit samples are sensitive to gamma-radiation, and were completely inhibited at 5.0 kGy radiation dose. The sensitivity of fungi to gamma-radiation has been established by Saleh and Aziz (1996) and Abd El-Aal and Aziz (1997) who recorded that the dose required for complete inhibition of fungi in different food and feed products ranged from 4 to 6 kGy. There are a number of reports which suggest that moulds are very sensitive to gamma-radiation and at mycotoxin production decreased after irradiation of foods (Refai et al., 1996; Youssef, Mahrous, & Aziz, 1999).

Table 6 shows that out of 100-unirradiated fruit samples stored for 28 days at refrigeration (below 10 °C), 81 samples were contaminated with mycotoxins. Penicillic acid and patulin were detected in 28 and 22 samples, respectively, at average concentration from 380 to 700 μg/kg. Aflatoxin B_1 and CPA were detected in 6 and 10 samples, respectively, at concentrations from 380 to 500 μg/kg, whereas ochratoxin A was detected in seven samples at concentrations from 400 to 600 μg/kg and citrinin was detected in eight samples at concentrations from 280 to 400 μg/kg. On the other hand from this table, it is clear that only 15 irradiated fruit samples at dose level 3.5 kGy were contaminated with mycotoxins. Aflatoxin B_1 was detected in one sample of plum and ochratoxin A in two samples of peach at a concentration of about 20 μg/kg. Also, penicillic acid was detected in four samples and patulin in eight fruit samples at concentrations from 10 to 50 μg/kg and there was no detection of CPA or citrinin in all irradiated fruit samples stored refrigerated for 28 days. There are a number of conflicting reports which suggest that the production of mycotoxins is either increased (Paster, Barkai-Golan, & Padova, 1985), decreased (Aziz et al., 1990) or unaffected (Paster & Bullerman, 1988) after irradiation of fungi under various laboratory conditions. It appears that the fungal strain, condition of storage, humidity and irradiation dose affect mould growth and toxin production (Mitchell, 1988). In this connection, the shelf life of various refrigerated fresh food may be extended to three weeks by applying low doses (less than 10 kGy) of ionizing radiation. The intention is to reduce the number of spoilage organisms and to alter their growth patterns (e.g., extension of lag phase) to enhance keeping quality (WHO, 1988). An irradiation dose of 1.5–3.0 kGy extends the storage life of refrigerated fresh strawberries by reducing the numbers of the principal spoilage fungi (species of *Botrytis*, *Rhizopus* and *Mucor*) (WHO, 1981). The expert committee that met in 1976 concluded that the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard, hence, toxicological testing of foods so treated is no longer required, also, a dose of 10 kGy introduces no special nutritional or microbiological problems (WHO, 1977).

Table 6
Detection of mycotoxins in different unirradiated and irradiated (3.5 kGy) fruit samples and stored at refrigeration for 28 days*

Samples	No. producing mycotoxins													
	Unirradiated							Irradiated at 3.5 kGy						
	No. of positive samples	Aflatoxin B_1	Cyclopi-azonic acid	Ochra-toxin A	Penicillic acid	Patulin	Citrinin	No. of positive samples	Aflatoxin B_1	Cyclopi-azonic acid	Ochra-toxin A	Penicillic acid	Patulin	Citrinin
Strawberry	10	–	3[a]	–	4[c]	3[c]	–	3	–	–	–	3**	–	–
Apricot	7	–	–	–	3[c]	4[c]	–	2	–	–	–	–	2**	–
Plum	10	−2[a]	–	–	4[c]	4[c]	–	1	1[d]	–	–	–	–	–
Peach	10	–	–	3[b]	6[c]	1[c]	–	2	–	–	2[d]	–	–	–
Grape	6	–	4[a]	–	–	–	2[e]	0	–	–	–	–	–	–
Date	4	4[a]	–	–	–	–	–	0	–	–	–	–	–	–
Fig	4	–	–	–	–	–	4[e]	0	–	–	–	–	–	–
Apple	10	–	–	–	6[c]	4[c]	–	3	–	–	–	–	3**	–
Pear	10	–	3[a]	–	1[c]	4[c]	2[e]	3	–	–	–	–	3**	–
Mulberry	10	–	–	4[b]	4[c]	2[c]	–	1	–	–	–	1**	–	–
Total	81	6	10	7	28	22	8	15	1	–	2	4	8	–

– Detection limits (μg/kg): aflatoxin $B_1 = 0.1$, ochratoxin A = 0.03, CPA = 0.1, penicilic acid = 0.05, patalin = 0.1, citrinin = 0.2.
– The procedure used proved valuable for screening the mentioned fruits for mycotoxins at a concentration 5 μg/kg (quantification limit, LQ).
* No. of samples examined = 100 samples, (–) no detection of mycotoxins.
** Approximately 10–50 μg/kg.
[a] Average conc. = 380–500 μg/kg.
[b] Average conc. = 400–600 μg/kg.
[c] Average conc. = 380–700 μg/kg.
[d] Traces approximately 20 μg/kg.
[e] Average conc. = 280–400 μg/kg.

In conclusion finding the mycotoxin-producing mould isolates in all the fruit types examined and the presence of mycotoxins in many of the fruit samples would seem to justify the development of analytical methods for the detection of these metabolites in other fruit products such as dried fruits and fruit concentrates.

The relationship of mycotoxins to food safety must be considered when methods of food preservation are being selected. Gamma-irradiation of foods has been proposed as a mean of food preservation, along with either sterilizing or pasteurizing doses.

It becomes essential to practice proper post-harvest handling, drying and storage of commodities. There is a need for disseminating knowledge on the harmful effects of mycotoxins and the possible dangers of consuming mouldy foods. Governments in different countries should establish monitoring agencies and set up laboratories for the surveillance of foods and feeds for mycotoxins.

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