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HPLC Detection of Aflatoxin in Meat, Poultry, and Fish and their Products and Detoxification by Gamma Radiation

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Abstract

One of the most desired and promising diets in Egypt is beef products. It is an enriched media for mycotoxins. The occurrence of aflatoxigenic *Aspergillus* species is inspected in commercial beef products, HPLC-quantitative amount of aflatoxin B1, B2, G1, and G2 excesses, and genetic identification of aflatoxin regulatory gene (*aflR1*) by conventional PCR. Two hundred and forty commercial products (minced meat, beef kofta, beef sausage, beef burger, beef luncheon, frozen meat, beef frozen liver, chicken luncheon, chicken burger, chicken frozen liver, mloha, and fesikh; n=20 for each) were collected from different markets at Aswan City, Egypt. Enumeration, isolation, and identification of mold species were carried to each sample. The amount of aflatoxins was measured using HPLC. Genetic identification of the *aflR1* gene in *Aspergillus* was performed using PCR. Mloha samples recorded the highest total mold count whereas the beef luncheon recorded the lowest mould count. Four fungal genera were identified and *Aspergillus* spp recorded the main with an incidence of 25.8%. By PCR, the *aflR1* gene was productively augmented in all the tested *Aspergillus* spp. The findings illustrated that among the samples that were examined; the prevalence of AFB1 was 65%, followed by AFG2 at 63%, AFB2 at 40%, and AFG1 at 30%. Additionally, mloha (724.2±14.6), poultry frozen liver (288±6.7), and beef frozen liver (91.6±12.2) had higher mean values of total aflatoxins contamination than other samples. Every sample that has been analyzed shows a positive correlation between the amount of reduced total mycotoxins found in the samples and the increased dose of gamma irradiation used to treat the samples. Conclusion: Aflatoxin is frequently linked to meat, poultry, and fish, as well as the products made from these foods. The production of aflatoxin in meat, as well as the products made from it, creates a danger to the public's health. Thus, the most effective way to prevent aflatoxigenic mould contamination during the product's production stages is to apply stringent hygienic standards when processing meat products and to use high-quality flavoring agents as spices.

KEYWORDS
Mycotoxin, Aflatoxin, *Aspergillus*, Meat-products, HPLC

INTRODUCTION

Meat and its products have a high nutritional value as they contain a high concentration of essential amino acids, vitamins, fats, and minerals, an ideal substrate for the growth of mycotoxigenic fungi, which leads to the formation of mycotoxin (Ferrão *et al*., 2017). Mold contamination of meat products can occur at various phases of the production process. It may happen when an animal is slaughtered in unsanitary conditions using tainted water, tools, and utensils when meat is processed and contaminated meat additives with mould spores are added, or when food is handled, packed, transported, or stored (Morshdy *et al*., 2015). Because it raises the possibility that these meat products will spoil and degrade, the danger of mould contamination in meat products is considered to be real. However, the most important factor contributing to food contamination by mould is the creation of mycotoxins. The more common and harmful types of mycotoxins are aflatoxins (AF), which are the main toxic secondary heterocyclic metabolites of some *Aspergillus* species like *A. parasiticus*, *A. flavus*, and *A. nomius* (Alcaide-Molina *et al*., 2009) that frequently contaminate human and animal food causing illness and mortality to consumers (Magnussen and Parsi, 2013). There are many kinds of Afs found in nature, but the B1, B2, G1, and G2 types are the most powerful (Shahbazi *et al*., 2017). Consumers are exposed to public health risks due to the mycotoxin contamination of beef and its products. Depending on the type and specific sites of the mycotoxins, various symptoms in people have been found, including liver cancer, hepatotoxicity, mutagenicity, nephrotoxicity, hormonal disruption, immunosuppression, and nervous system disturbance (Gagaoua and Boudechicha, 2018). Food radiation has the potential to increase food's shelf life while preserving its organoleptic and safety qualities. The Codex Alimentarius Commission has authorized gamma, electron beam (E-beam), and X-ray as the three ionizing sources for food radiation (Iqbal *et al*., 2013). A quick and efficient decontamination method for food protection is gamma irradiation. Without sacrificing the quality and safety of food, this method effectively eliminates pathogenic and spoilage microorganisms and reduces some toxins, such as

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mycotoxins (Domijan *et al*., 2015). Gamma radiation is known to have a direct effect on mycotoxins by degrading them and an indirect effect by inhibiting or delaying fungal development in mycotoxicology (Calado *et al*., 2018). The current study's objectives were to quantify aflatoxin levels in various meat, poultry, and fish products using an HPLC assay, identify the aflatoxin regulatory gene (*aflR1*) in contaminated products using a conventional PCR assay, and investigate the detoxification of aflatoxin levels by gamma radiation.

MATERIALS AND METHODS

Mould Assessment

Samples collection

A total of 240 samples were collected in Aswan City, representing minced meat, beef burger, beef kofta, beef sausage, beef luncheon, frozen meat, beef frozen liver, chicken luncheon, chicken burger, chicken frozen liver, mloha, and fesikh. The samples were collected in an aseptic manner in sterile plastic bags and transferred to the lab in a box of ice for mycological examination and aflatoxin residue detection.

Sample preparation

Each sample was combined with 225 Ml of sterile peptone water (0.1%) to encourage the development of mould involved with the samples under investigation (Downes and Ito, 2001).

Assessment of the total mould count (APHA, 1985)

The produced dilutions were inoculated onto Oxoid Dichloran Rose Bengal Chloramphenicol agar (UK). The inoculation plates were checked for mould growth and enumeration as CFU/g after being cultured for up to 5 days at 25°C.

Identification of Mould isolates

The identification of suspected colonies as previously described by Pitt and Hocking (2009)

Identification of aflatoxin regulatory gene (aflR1) in isolated Aspergillus spp. By conventional PCR assay (Bintvihok et al., 2016)

For DNA extraction, phenotypically colonies were used. Using Quick-DNA™ Fungal/Bacterial MiniPrep Extraction kit (Zymoresearch, Cat. No. D6005). Using the forward primer *AflR*-1F 5′- ACCGCATCCACAATCTCAT-3′ and the reverse primer *AflR*-2R 5′- GTGCAGTTCGCTCAGAACA- 3′ (Willowfort Co., United Kingdom), ten separated *Aspergillus* were subjected to PCR amplification for the *aflR1* gene, with an actual product size of 800 bp.

Quantitative assessment of aflatoxins residues (B1, B2, G1, and G2) by HPLC

Standard materials solutions

Verified reference acetonitrile solutions with aflatoxins standards (B1, B2, G1, and G2) at a concentration of 3g/ml provided by Sigma-Aldrich Co. The Association of Official Analytical Chemists (AOAC, 2000) technique was followed in the preparation of the stock standard solution and working standard solutions.

Sample preparation for residue analysis

To prepare for the assay, partially thaw frozen tissues for 30 minutes at room temperature (23°C) and blend for 20 to 30 seconds at high speed in a blender to create a uniform paste-like consistency.

Separation and cleanup (Brera *et al*., 2011)

Fill a polypropylene container with 5g of weight. 25mL of methanol (80%) and 0.5 g of sodium chloride should be added. For three minutes, mix at a fast speed. Through filter paper, sieve the extract. Dilute 3 mL of PBS with 3 mL of filtrate. Completely combine. At 10,000 rpm Centrifuge the diluted material for 10 minutes. Adding 4mL of the diluted material to the conditioned immunoaffinity column (IAC) followed by 1mL of PBS. In two steps, elute mycotoxins: Apply 1.0 mL of methanol to the IAC first, and then let it pass through naturally. Collect Elute in a volumetric beaker with a 5 mL calibration. Wait 1 minute before applying a second amount of 1.0 mL methanol. To gather the last few drops, blow air through the column. Before analyzing the sample, keep it at +4°C in a 5mL volumetric flask filled to the mark with DW and thoroughly mixed.

Derivatization (AOAC, 1995)

A post-column LC pump (Zero-dead volume T piece, reaction tubing minimum 450 × 0.5 mm Diameter in PTFE) (LC pump Lab flow 2000, Labservice Analytica, Bologna, Italy) is used to perform pre-column derivatization using a 0.005% aqueous solution of Pyridine Hydrobromide Perbromide (PBPB) at a flow rate of 0.4 mL/min.

Analytical method validation

The procedure was approved in accordance with ICH, 2005 standards:

Limit of detection (LOD) and limit of quantification (LOQ)

They were determined LOD = 3.3S/band depending on a standard deviation of intercept (S) and slope (b). LOQ = 10S/b

HPLC analysis conditions

Agilent HPLC equipment was used to analyze the AF (Quaternary pump, autosampler model 1200, USA). a pre-packaged Agilent LiChrospher C18 column measuring 250 mm, 4.6 mm in diameter, and 5 m in particle size. A post-column LC pump (zero-dead volume T piece, reaction pipe minimum 450 × 0.5 mm id in PTFE) (LC pump Lab flow 2000, Lab service Analytica, Bologna, Italy). Fluorescence Detector Jasco FP1520 (Jasco Corporation, Tokyo, Japan). The activation wavelength was 365 nm, and the emission wavelength was 442. A program developed by Agilent in the USA for automated data analysis.

Irradiation's effects on the reduction of mycotoxins (Aziz et al., 2004)

In three replications, high-positive product samples were put in polyethylene pouches and subjected to doses of 6, 8, and 20 Katy using 60CO gamma rays (Gamma Cell mould 220 apparatus, NCRRT, Nasr City, Cairo, Egypt).

Statistical analysis

According to Feldman *et al*. (2003), the obtained findings were statistically analyzed by application of the Analysis of Variance (ANOVA) test. Log10 reduction and reduction percentages were determined using Excel software (George Brown, 2017).
log10 reduction = (A) - (B)
Where A=log10 viable microbes number before the examination.
B is the log10 living microbe's count after the examination.
Log reduction % = (A - B) /A x 100
If the number has become negative, both the number and the percent have increased by a log10 factor.

RESULTS

The occurrence of mold in examined samples (Table 1) was found to be 10^2 (42.5%) out of 240 samples distributed as follows: 8 (40%), 6 (30%), 6 (30%),12 (60%), 12(60%), 11 (55%), 4 (20%), 8 (40%), 6 (30%), 12 (60%),14 (70%) and 3 (15%) with the mean value of mould count of $3.6\times10^2\pm0.2\times10^2$, $2.03\times10^2\pm4\times10^2$, $1.51\times10^2\pm3.03\times10^2$, $3.45\times10^2\pm0.3\times10^2$, $3.15\times10^2\pm1.2\times10^2$, $3.42\times10^2\pm0.43\times10^2$, $1.33\times10^2\pm0.05\times10^2$, $1.35\times10^2\pm1.9\times10^2$, $0.85\times10^2\pm0.6\times10^2$, $0.95\times10^2\pm0.04\times10^2$, $4.7\times10^2\pm1.2\times10^2$, and $0.87\times10^2\pm0.05\times10^2$ for minced meat, beef burger, beef Luncheon, beef kofta, beef sausage, beef frozen liver, frozen meat, chicken burger, chicken luncheon, chicken frozen liver, mloha, and fesikh, respectively. There is a statistically significant variation in the mould count between the different samples that were examined (P< 0.0001).

Table 2 shows the incidence of isolated mould species that *Aspergillus*, *Penicillium*, *Cladosporium*, and *Geotrichum candidum* could be isolated from 62 (25.8%), 14 (5.83%), 20 (8.33%), and 3 (1.3%) out of 240 different samples, respectively.

Aspergillus species recognized in the examined product (Table 3) were *A. niger*, *A. carbonarius*, *A. parasiticus*, *A. versicolor*, *A. fumigatus*, *A. flavus*, *A. alutacus*, *A. Aegypticus*, *A. Flavofurcatus*, and *A. Sulphurus* with the incidence of 12.5%, 9.6%, 5.42%, 9.2%, 1.3%, 3.8%, 0.83%, 1.7%, 0.83%, and 1.3%, respectively. In addition, the outcomes of the conventional PCR analysis revealed that the DNA extract of the *Aspergillus* isolates has the aflatoxin regulatory gene (*aflR1*) (Figure 1).

Regarding the presence of AFB1, AF B2, AFG1, and AFG2 in the analyzed samples, only 39 (65%), 24 (40%), 18, 30, 38 (63%), and 73.3 (26.7%) samples, respectively, allowed for their detec-

Table 1. Occurrence and total mold count of the examined products (n.=20 each).

Examined samples	Positive sample		Total mold count			*P*-value
	No.	%	Minimum	Maximum	Mean±SE	
Minced meat	8	40	0.1×10^2	7.1×10^2	$3.6\times10^2\pm0.2\times10^{2a}$	0.01
Beef burger	6	30	1.15×10^2	3.8×10^2	$2.03\times10^2\pm4\times10^{2b}$	0.07
Beef luncheon	6	30	0.5×10^2	2.75×10^2	$1.51\times10^2\pm3.03\times10^{2c}$	0.01
Beef kofta	12	60	0.2×10^2	7×10^2	$3.45\times10^2\pm0.3\times10^{2a}$	0.01
Beef sausage	12	60	0.4×10^2	6.13×10^2	$3.15\times10^2\pm1.2\times10^{2a}$	0.01
Beef frozen liver	11	55	1.17×10^2	6.8×10^2	$3.42\times10^2\pm0.43\times10^{2a}$	0.02
Frozen meat	4	20	0.7×10^2	2×10^2	$1.33\times10^2\pm0.05\times10^{2c}$	0.02
Chicken burger	8	40	0.01×10^2	6.05×10^2	$1.35\times10^2\pm1.9\times10^{2bc}$	0.09
Chicken luncheon	6	30	0.2×10^2	1.75×10^2	$0.85\times10^2\pm0.6\times10^{2c}$	0.01
Chicken frozen liver	12	60	0.5×10^2	2.3×10^2	$0.95\times10^2\pm0.04\times10^{2c}$	0.03
Mloha	14	70	1.5×10^2	7.9×10^2	$4.7\times10^2\pm1.2\times10^{2d}$	0.08
Fesikh	3	15	0.6×10^2	1.2×10^2	$0.87\times10^2\pm0.05\times10^{2c}$	0.02
Total No.	102			42.50%		

p≤0.0014, considered extremely significant. Mean values with the same letters in each column are not significant difference (p≥0.05) using ANOVA test.

Table 2. Occurrence of mold genera in examined products (n.=20).

Examined samples	Mold spp.								Total spp	
	Aspergillus spp		*Penicillium* spp		*Cladosporium* spp		*Geotrichum candidum*			
	No.	%	No.	%	No.	%	No.	%	No.	%
Minced meat	6	30	3	15	4	20	0	0	13	65
Beef burger	6	30	4	20	2	10	2	10	14	70
Beef luncheon	5	25	0	0	0	0	1	5	6	30
Beef kofta	12	60	3	15	0	0	0	0	15	75
Beef sausage	6	30	0	0	1	5	0	0	7	35
Beef frozen liver	4	20	0	0	3	15	0	0	7	35
Frozen meat	3	15	1	5	2	10	0	0	6	30
Chicken burger	8	40	0	0	3	15	0	0	11	55
Chicken luncheon	4	20	2	10	2	10	0	0	8	40
Chicken frozen liver	2	10	0	0	1	5	0	0	3	15
Mloha	3	15	1	5	1	5	0	0	5	25
Fesikh	3	15	0	0	1	5	0	0	4	20
Total No.	62	25.8	14	5.83	20	8.33	3	1.3	99	41.3

Table 3. Incidence of identified *Aspergillus* species in examined products (n.= 20).

Examined samples	*Aspergillus* species (%)																			
	A. niger		*A. carbonarius*		*A. parasiticus*		*A. versicolor*		*A. fumigatus*		*A. flavus*		*A. alutacus*		*A. aegypticus*		*A. flavofurcatus*		*A. sulphurus*	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Minced meat	2	10	1	5	1	5	7	35	-	-	-	-	-	-	-	-	-	-	-	-
Beef burger	4	20	5	25	-	-	3	15	-	-	3	15	-	-	-	-	-	-	-	-
Beef luncheon	3	15	5	5	3	15	2	10	-	-	1	5	-	-	-	-	-	-	-	-
Beef kofta	2	10	9	45	-	-	-	-	3	15	-	-	-	-	-	-	-	-	-	-
Beef sausage	3	15	2	10	-	-	2	10	-	-	-	-	-	-	4	20	-	-	1	5
Beef frozen liver	4	20	-	-	-	-	1	5	-	-	2	10	-	-	-	-	-	-	-	-
Frozen meat	3	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	10	-	-
Chicken burger	2	10	1	5	1	5	7	35	-	-	-	-	-	-	-	-	-	-	-	-
Chicken luncheon	3	15	-	-	-	-	-	-	-	-	1	5	2	10	-	-	-	-	-	-
Chicken frozen liver	-	-	-	-	1	5	-	-	-	-	-	-	-	-	-	-	-	-	2	10
Mloha	3	15	-	-	5	25	-	-	-	-	2	10	--	-	-	-	-	-	-	-
Fesikh	1	5	-	-	2	10	-	-	-	-	-	-		-	-	-	-	-	-	-
Total	30	12.5	23	9.6	13	5.42	22	9.2	3	1.3	9	3.8	2	0.83	4	1.7	2	0.83	3	1.3

Table 4. Occurrence of aflatoxin residues in examined products samples and their acceptability according to FAO (2004)*.

Examined samples		Positive sample								Acceptable sample %		Non acceptable sample %	
		AF B1		AF B2		AF G1		AF G2					
	No	No	%	No	%	No	%	No	%	No	%	No	%
Minced meat	5	3	60	3	60	3	60	4	80	5	100	0	0
Beef burger	5	4	80	0	0	0	0	4	80	5	100	0	0
Beef luncheon	5	3	60	0	0	0	0	3	60	5	100	0	0
Beef kofta	5	5	100	3	60	3	60	5	100	5	100	0	0
Beef sausage	5	5	100	4	80	4	80	5	100	3	60	2	40
Beef frozen liver	5	3	60	3	60	3	60	3	60	1	20	4	80
Frozen meat	5	2	40	0	0	0	0	2	40	5	100	0	0
Chicken burger	5	4	80	4	80	0	0	4	80	5	100	0	0
Chicken luncheon	5	3	60	3	60	3	60	3	60	5	100	0	0
Chicken frozen liver	5	3	40	2	40	2	40	2	40	0	0	5	100
Mloha	5	3	60	2	40	0	0	3	60	0	0	5	100
Fesikh	5	1	20	0	0	0	0	0	0	5	100	0	0
Total	60	39	65	24	40	18	30	38	63	44	73.3	16	26.7

*Regulator limits (>20g/kg) for meat products.

Table 5. Concentration of aflatoxin residues (ppb) in examined products.

Examined samples	Mean (±S.E) of aflatoxin detected				
	B1	B2	G1	G2	Total Afs
Minced meat	0.73±0.2^{a}	0.9±0.3^{a}	0.62±0.2^{a}	**0.74±0.2**^{a}	**3.9±0.28**^{a}
Beef burger	**1.4±0.3**^{b}	**0**	**0**	**3.02**±0.7^{b}	**5.4±0.13**^{b}
Beef luncheon	**2.6±0.05**^{b}	**0**	**0**	**2.12**±0.02^{b}	**5.14**±0.18^{b}
Beef kofta	**2.4**±0.08^{b}	**1.18**±0.06^{a}	**0.74±0.03**^{a}	**1.92**±0.3^{a}	**5.68**±0.2^{b}
Beef sausage	**5.03±0.26**^{c}	**1.76**±0.05^{a}	**1.02**±0.05^{a}	**1.73±0.08**^{a}	**9.85**±0.64^{b}
Beef frozen liver	32.3±2.3^{d}	**8**±0.2^{b}	**6.6**±0.2^{b}	**43.3**±4.6^{c}	**91.6**±12.2^{c}
Frozen meat	**1.16**±0.09^{b}	**0**	**0**	**1.66**±0.2^{b}	**2.82±0.25**^{a}
Chicken burger	**0.65±0.001**^{a}	**1.66±0.03**^{a}	**0**	**0.7**±0.03^{a}	**2.89±0.9**^{a}
Chicken luncheon	**1.6±0.01**^{b}	**0.68**±0.002^{a}	**0.4**±0.01^{a}	**2.3±0.3**^{b}	**5.3**±0.73^{b}
Chicken frozen liver	**122.3±2.7**^{e}	**23.3**±1.2^{c}	**21**.0±**1**.0^{e}	**102**.0±**1.6**^{d}	**288**.0±**6.7**^{c}
Mloha	**392**.0±5.8^{f}	**189**.0±**2.2**^{d}	**0**	**258**.0±**4**.0^{d}	**724.2**±14.6^{d}
Fesikh	**1**.0±0.001^{b}	**0**	**0**	**0**	**1**.0±0.001^{a}

P≤ 0.05 considered extremely significant. Mean values with the same letters in each column are not significant difference using ANOVA test.

tion and quantification (Table 4). According to the regulatory authority (FAO, 2004), all samples approved for their aflatoxin residues exceeded the legal and regulatory limits (>20 g/kg) for meat products, except chicken frozen liver, mloha, and only 80% beef frozen liver and 40% beef sausage samples. The data in Table 5, demonstrates the frequency of aflatoxin remnants (ppb) in various product samples with a mean value of 0.73±0.2, 1.4±0.3, 2.6±0.05, 2.4±0.08, 5.03±0.26, 32.3±2.3, 1.16±0.09, 0.65±0.001, 1.6±0.01, 122.3±2.7, 392±5.8 and 1±0.001 for AFB1, 0.9±0.3, 0, 0, 1.18±0.06, 1.76±0.05, 8±0.2, 0, 1.66±0.03, 0.68±0.002, 23.3±1.2, 189±2.2 and 0 for AF B2, 0.62±0.2, 0, 0, 0.74±0.03, 1.02±0.05, 6.6±0.2, 0, 0, 0.4±0.01, 21±1, 0 and 0 for AFG1 and 0.74±0.2, 3.02±0.7, 2.12±0.02, 1.92±0.3, 1.73±0.08, 43.3±4.6, 1.66±0.2, 0.7±0.03, 2.3±0.3, 10^2±1.6,258±4 and 0 for AFG2. The difference relating to the meat products examined sample was extremely significant ($p \leq 0.05$). The aflatoxin residues in various products following gamma irradiation are shown in Table 6, together with the amount of total aflatoxin reduced by increasing the dose of gamma irradiation, and the findings demonstrate a positive correlation between them in all samples analyzed.

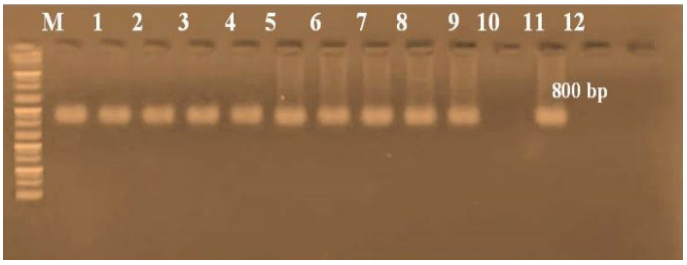

Fig. 1. Agarose gel electrophoresis of (*aflR1*) of *Aspergillus* spp., Lane M: DNA ladder, Lane 1-10: positive for the presence of (*aflR1*) in *Aspergillus* spp., Lane11: Negative control, and Lane 12: Positive control.

DISCUSSION

Most documented outbreaks of food poisoning are primarily caused by meat and meat products. Consequently, it is essential to use bacteriological measures to assess those goods' value (Abuzaid *et al*., 2020). Therefore the occurrence of mold in different inspected products was recorded at 42.5% (Table 1) in which the highest Aflatoxigenic fungi incidence was found in mloha (70%), followed by beef kofta, beef sausage, chicken frozen liver (60% for each), beef frozen liver (55%), minced meat and a chicken burger (40% each), beef burger, beef luncheon, and chicken luncheon (40% each), frozen meat (20%) whereas the lowest prevalence of Aflatoxigenic fungi were found in Fesikh (15%). Soliman *et al.* (2019) got similar findings in Egypt and reported that various mould spp. was present in 60% and 67.5% of

Table 6. Reduction percentage of aflatoxin (%) in meat and their products by using gamma irradiation.

Examined samples	Irradiation dose (kGy)	Type of aflatoxins														
		B1			B2			G1			G2			TAF		
		B (ppb)	A (ppb)	R %	B (ppb)	A (ppb)	R %	B (ppb)	A (ppb)	R %	B (ppb)	A (ppb)	R %	B (ppb)	A (ppb)	R %
Minced meat	6	2.2	1.83	16.81	1.9	1.24	4.73	1.2	0.92	23.33	1.4	1.12	20	6.7	5.11	23.73
	8		1.52	30.9		0.98	448.42		0.78	35		0.87	37.85		4.15	38
	10		1.12	49.09		0.76	60		0.62	48.33		0.62	55.71		3.12	3.97
	20		0.85	61.36		0.58	69.47		0.53	55.83		0.55	60.7		2.51	62.53
Beef burger	6	2.5	2.1	16	-	-	-	-	-	-	5.6	4.97	11.25	8.1	7.07	12.7
	8		1.87	25.2		-	-		-	-		3.88	30.7		5.75	29
	10		1.44	42.4		-	-		-	-		2.53	54.82		3.97	50.98
	20		1.1	56		-	-		-	-		2.1	62.5		3.2	60.49
Beef luncheon	6	4.37	4.2	3.8	-	-	-	-	-	-	2.76	2.31	16.3	7.04	6.51	7.52
	8		3.74	14.41		-	-		-	-		1.95	29.34		5.69	19.17
	10		3.2	26.77		-	-		-	-		1.42	48.55		4.62	34.37
	20		2.15	50.8		-	-		-	-		0.96	65.21		3.11	55.82
Beef kofta	6	5.3	4.94	6.79	3.5	3.1	11.14	1.3	0.97	25.38	3.1	2.86	7.74	13.2	11.87	10.07
	8		4.27	19.43		2.82	19.42		0.74	43.07		2.27	26.77		10.1	23.48
	10		3.92	26.03		2.34	33.14		0.66	49.23		1.83	40.96		8.75	33.7
	20		2.47	53.39		1.89	46		0.54	58.46		1.47	52.58		6.37	51.74
Beef sausage	6	15	14.23	5.13	5.7	5.21	8.5	3.1	2.88	7.09	4,9	4.2	14.28	28.7	26.52	7.5
	8		13.22	11.86		4.48	21.4		2.1	32.25		3.51	30.88		23.31	18.78
	10		12.48	16.8		3.82	32.98		1.67	46.12		2.86	41.63		20.83	27.42
	20		11.58	22.8		2.86	49.82		1.18	61.93		1.92	60.8		17.54	38.88
Beef frozen liver	6	46	44.73	2.76	13	12.3	5.38	11	10.2	7.27	52	47.2	9.2	122	114.43	6.2
	8		43.88	4.6		11.1	14.61		9.48	13.8		38.54	25.88		103	15.57
	10		41.72	9.3		10.79	17		7.88	28.36		28.64	44.9		89.03	27.02
	20		40.36	12.26		9.34	28.15		6.35	41.54		22.42	56.88		78.47	35.68
Frozen meat	6	1.4	1.1	21.42	-	-	-	-	-		2.2	1.94	11.8	13.9	3.04	78.12
	8		0.89	36.42		-	-		-	-		1.62	26.36		2.51	81.94
	10		0.67	45.71		-	-		-	-		1.3	40.9		1.97	85.82
	20		0.42	7900		-	-		-	-		0.97	78.12		1.39	90

(B. Aflatoxins concentration before treatment. (A. Aflatoxins concentration after treatment. (R %. Reduction %.

Table 7. Reduction percentage of aflatoxin (%) in chicken and fish products by using gamma irradiation.

Examined samples	Irradiation dose (kGy)	Type of aflatoxins														
		B1			B2			G1			G2			TAF		
		B (ppb)	A (ppb)	R %	B (ppb)	A (ppb)	R %	B (ppb)	A (ppb)	R %	B (ppb)	A (ppb)	R %	B (ppb)	A (ppb)	R %
Chicken burger	6	1.2	0.97	9.16	2.3	1.98	13.9	-	-	-	1.05	0.98	6.6	4.05	3.93	2.96
	8		0.74	38.33		1.57	31.7		-	-		0.84	20		3.15	22.22
	10		0.57	52.5		1.2	47.8		-	-		0.63	40		2.4	40.74
	20		0.39	67.5		0.97	57.8		-	-		0.58	44.76		1.94	52
Chicken luncheon	6	2	1.68	16	1.1	0.96	12.7	0.7	0.62	11.42	2.9	2.44	15.86	6.7	5.7	14.92
	8		1.43	28.5		0.76	30.9		0.54	22.85		1.89	34.82		4.62	31
	10		0.98	51		0.66	40		0.44	37.14		1.67	42.4		3.75	44
	20		0.78	61		0.53	51.8		0.39	44.71		1.38	52.4		3.08	55.52
Chicken frozen liver	6	150	141	6	43	36.4	15.3	22	20.79	5.5	118	106	10.16	333	304.19	8.65
	8		110.3	26.4		32.48	24.46		17.38	21		97.47	17.39		257.63	22.63
	10		100.4	33		26.41	38.58		13.54	38.45		77.42	34.38		217.7	34.6
	20		70.3	53.1		19.64	54.3		9.34	57.54		58.63	50.3		157.9	52.57
Mloha	6	500	462	7.6	230	204	11.3	-	-	-	10	217	30	1040	883	15
	8		415.26	16.9		184	20		-	-		145	53.22		744.26	28.43
	10		340	32		159.6	30.6		-	-		25	91.9		524.6	49.55
	20		187	62.6		87	62.17		-	-		12.1	96		286.1	72.49
Fesikh	6	4	3.61	9.75	-	-	-	-	-	-	-	-	-	4	3.61	9.75
	8		3.32	17		-	-		-	-		-	-		3.32	17
	10		2.88	28		-	-		-	-		-	-		2.88	28
	20		2.42	39.5		-	-		-	-		-	-		2.42	39.3

(B. Aflatoxins concentration before treatment. (A. Aflatoxins concentration after treatment. (R %. Reduction %.

the luncheon and hamburger samples, respectively. While Abuzaid *et al*. (2020) separated 62.5% and 82.5% from Kofta and sausage samples, Abd El-Tawab *et al*. (2020) guaranteed that moulds were divorced from 50% and 80% of the examined minced beef and sausage. The rate of moulds in minced beef meat, beef burger, sausage, beef luncheon, and kofta was 50%, 65%, 77%, 60%, and 50%, respectively, according to higher findings published by Abuelnaga *et al*. (2021). However, it was less frequent than the 93.3% recorded by Hussein (2008) for the prevalence of mould in kofta.

The highest mould count (CFU/g) in the present study was in mloha ($4.7\times10^2\pm1.2\times10^2$), whereas the beef luncheon recorded the lowest mould counts with a mean value of $0.85\times10^2\pm0.6\times10^2$. The mean values of total mould counts (TMC/g) in various meat product samples are shown in Table 1. On the other hand, a statistically significant variance in the mould count between the different specimens that were examined ($p\leq0.0014$). Similar findings were made by Elsayed *et al*. (2018), who discovered that the mean mould counts in luncheon and minced meat were $1.3\times10^2\pm2.1\times10$ cfu/g and $2.8\times10^2\pm4.3\times10$ cfu/g, respectively, while the finding for sausage was lower at $6.9\times10^2\pm1.2\times10^2$ cfu/g. In addition, Abuelnaga *et al*. (2021) found comparable outcomes in sausage ($3.4\times10^2\pm2.1\times10^2$ cfu/g), but higher results in luncheon ($2.3\times10^2\pm1.3\times10^2$), while lower results in minced meat ($2.5\times10^2\pm1.6\times10^2$ cfu/g), beef burger ($1.3\times10^3\pm9.2\times10^2$ cfu/g), and kofta ($0.5\times10\pm0.1\times10$), respectively. A lower mold count was achieved by Abuzaid *et al*. (2020) with mold counts of $1.1\times10^3\pm0.14\times10^3$ and $1.4\times10^3\pm0.27\times10^3$ in sausages and Kofta, respectively. As well lower results reported by El-Tabiy (2006) and Algammal *et al*. (2021) in sausage samples were $2.26\times10^2\pm0.58\times10^2$ CFU/g and $2.9\times10^2\pm0.91\times10^2$ CFU/g, respectively. These variations were ascribed to changes in the quantity and kinds of additives used in the production of meat products, as well as changes in the products' exposure to temperatures and time and the sanitary measures used during processing.

Table 2 lists all the mycoflora that were isolated from the samples of evaluated products. The findings revealed the identification and recording of four fungal species. These include *Geotrichum candidum*, *Aspergillus* spp., *Penicillium* spp., and *Cladosporium* spp. *Aspergillus* spp. was the most frequently isolated mould species from samples, with a prevalence of 25.8%, followed by *Cladosporium* spp., 8.33%, and *Penicillium* spp., 5.83%, while *Geotrichum candidum* had a lesser prevalence of 1.3%. The *Aspergillus* species produce a class of secondary fungal metabolites known as aflatoxins.

In the current study, the most predominant species are *A. niger*, *A. carbonarius*, *A. parasiticus*, *A. versicolor*, *A. fumigatus*, *A. flavus*, *A. alutacus*, *A. Aegypticus*, *A. Flavofurcatus*, *A. Sulphurus* with the prevalence of 12.5%, 9.6%, 5.42%, 9.2%, 1.3%, 3.8%, 0.83%, 1.7%, 0.83%, and 1.3%, respectively (Table 3). In contrast to earlier research, nine fungal species, *Aspergillus*, *Penicillium*, *Cladosporium*, *Epicoccum, Phoma, Geotrichum,* and *Trichoderma Paecilomyces* were discovered and documented by Marwa *et al*. (2018). *Penicillium* was the most frequently found fungus in processed beef samples, accounting for 89.71%, followed by *Aspergillus* 4.59% (*A. flavus* 2.34%, *A. niger* 1.22%, and *A. parasiticus* 0.94%), Geotrichum spp 2.91%, *Cladosporium* spp 1.50%, *Phoma* spp. 0.56%, *Paecilomyces* spp. 0.47%, and *Epicoccum* spp. 0.19% were next in order of fungal frequency linked with processed meat samples. *Trichoderma* sp. and *Alternaria* sp. had a 0.09% lower fungal incidence. These results are less significant than those published by Ismail *et al*. (2013), who claimed that meat products could be used to identify 7 mould genera. *Aspergillus*, *Penicillium*, *Eupencillium, Eurotium, Mucor, Cladosporium*, and *Byssochlamysnivea* were the mould genera that were discovered. The frequency of isolated mould taxa was *Penicillium* (26.3%), followed by *A. flavus* (18.4%), *P. corylophilum* (18.4%), *Mucor* (13.1%), *Byssochlamysnivea* (2.6%), and 5.3% for each of the *Cladosporium* species, *P. simplicissimum*, *P. digitatum*, and *Eupencillium* species. *Aspergillus* spp. had the highest incidence rate (49%) followed by *Penicillium* (34%), *Cladosporium* and *Alternaria* (15%), *Acremonium* (12), *Rhizopus* (10%), *Rhizomucor* (8%), Absidia (3%) and *Chrysosporium* (2%), higher results by Morshdy *et al*. (2015) identified nine mould genera from the beef burger,

luncheon, Kofta, and sausage. From samples of inspected meat products, five species of *Aspergillus* could be isolated. The most frequent species were *A. niger* (22%), *A. flavus* (16%), *A. fumigatus* (12%), *A. parasiticus* (2%), and at least *A. ochraceus* (1%). This variability in fungal species could be brought on by the use of various media kinds and storing temperatures. Humidity, water activity (aw), pH, temperature, environment, nutrients, nature of the substrate, fungal load, physiological state, and microbial interaction are just a few of the many variables influencing both the development of various types of moulds and their synthesis for mycotoxins. Molds frequently got access to products made of preserved meat, which became active as a result of the prolonged ripening time of these types of processed meat, which led to the production of mycotoxins (Chen *et al*., 2020).

The traditional approaches to identifying and detecting fungi in foods and feeds focus on phenotypic features, and the results may vary considerably different based on the culture conditions and media used. In addition, they take a lot of time, effort, and knowledge from mycologists. Recently, PCR-based techniques have become important instruments for identifying fungi that produce aflatoxin in foods (Rodriguez *et al*., 2012). As a result, in the current research, the PCR amplification of the aflatoxin regulatory gene (*aflR1*) and macroscopic and microscopic examinations were used to corroborate the diagnosis of the retrieved *Aspergillus* isolates. Additionally, PCR was effectively used to amplify the *aflR1* gene in *Aspergillus* isolates (Figure 1). Aflatoxin production is primarily regulated by the aflatoxin regulatory gene (*aflR*). The functional genes regulating the aflatoxin production pathway are activated by the *AflR* protein, which is expressed by the *aflR* gene (Wang *et al*., 2011). The current result was in agreement with that obtained by Cruz and Buttner (2008); Mahmoud (2015) and Bintvihok *et al*. (2016) found that examined isolates expressed a regulatory alfR gene. According to Awad *et al*. (2019), there is a positive correlation between the quantity of aflatoxins produced by isolates and the mean expression level of regulatory genes. The found variations in aflatoxins production capacity could be attributed to variations in the expression of aflatoxins regulatory and biosynthetic genes among toxigenic isolates from tested products as a result of variations in environmental conditions. The HPLC technique, which depends on the chemical and physical characteristics of mycotoxins, is the most well-established assay for estimating the number of aflatoxins in food. It offers an accurate, precise, and specific way to determine the aflatoxins concentrations in tainted food. Detection of aflatoxins by HPLC in examined products was documented in Table 4, whereas aflatoxin residues were presented in Table 5. The data revealed that the prevalence of AFB1 was 65% among examined samples followed by 63% for AFG2, 40% for AFB2, and 30 for AFG1. Furthermore, minced meat, beef kofta, beef sausage, beef frozen liver, chicken luncheon, and chicken frozen liver consists of AFB1, AFB2, AFG1, and AFG2. Mloha besides chicken burgers consists of aflatoxin AFB1, AFB2, and AFG2 while beef burgers, beef luncheon, and frozen meat consist of aflatoxin AFB1 and AFG2, and Fesikh consists of aflatoxin AFB1 only. Meanwhile, the total aflatoxins mean value was higher in mloha (724.2±14.6), chicken frozen liver (288±6.7), and beef frozen liver (91.6±12.2) than in other samples. The obtained results showed that the amount of total aflatoxin found in chicken frozen liver and mloha, as well as 80% beef frozen liver and 40% beef sausage samples, exceeded the international regulatory limits (>20g/kg) for meat products (FAO, 2004), whereas other products were within the allowable limits (Table 4). Because of the average concentration of total aflatoxin (ppb), there was an extremely significant difference between the examined sample and the other ($p<0.05$). As the liver is the harbor site for mycotoxins residues, the findings indicate that the highest concentration of mycotoxins under examination was found there. It is significant to note that, when compared to other kinds of aflatoxins, AFB1 is the most potently carcinogenic even at very low concentrations (WHO, 2002). Higher results reported by Shaltout *et al*. (2014) who recorded that AFB1 concentration (µg/kg) in kofta, sausage, and luncheon was 13.38±1.52, 9.03±1.17, and 8.8±0.95, 8.50±0.7, 5.20±0.69, and 5.57±0.72 for AFB 2 (µg/kg), 4.76±0.83, 3.35±0.49, and 3.84±0.58for AFG1 (µg/kg) and 3.18±0.52, 2.33±0.29 and 2.50±0.03, for AFG 2 (µg/kg), respectively. As well Morshdy *et al*. (2015) reported higher mean values of AFB1, AFB2, and AFG1 (2.14±0.35, 2.88±1.08, 0.48±0.15 ppb) and lower values of AFG2 (0.17±0.05 ppb) in the examined beef burger. While the lower mean value for sausage was 0.69±0.22, 0.31±0.15, 0.62±0.28 and 0.16±0.05 ppb, respectively and the mean value for kofta samples was 0.42±0.11, 1.60±0.39, 0.67±0.30 and 0.57±0.14 ppb, respectively. Shabana *et al*., (2008) obtained nearly similar kofta for AFB1 of 6.70±0.89 but a lower concentration of AFG1 of 4.76. As well nearly similar results for AFB1in the beef product (0. 15 to 6.36) reported by Herzallah (2009) and Ali *et al*. (2005) who recorded 1.53µg/kg in luncheon, and Altalhi and Albashan (2004) who reported 3.6µg/kg in frozen meat but results were higher than reported that by Ismail *et al*. (2013) who reported AFB1was 10.4±5.1 in luncheon. Hassanin *et al*. (2016) observed that luncheon recorded 0.98 ppb and chicken burger recorded 1.63 ppb. Furthermore, Markov *et al*. (2013) revealed that AFB1 was present in 10% of the tested samples, with the highest AFB1 level of 3.0 mg/kg. Additionally, EL-Mossalami (2010) recorded 0.87, 0.41, and 0.59 µg/kg of FB1residues in frozen meat, luncheon, and beef burger samples also Awad *et al*. (2019) reported lower residues in the chicken liver with the mean value of 5.37±1.53. Iqbal *et al*. (2014) found total aflatoxins in the chicken livers with a mean value of 3.23±0.82 µg/kg.

Modern food preservation techniques include food radiation, a physical procedure that uses ionizing radiation to stop the development of undesirable biological organisms or to decrease their population (Ferreira-Castro *et al*., 2007). International organizations like the World Health Organization (WHO), the Food and Agriculture Organization (FAO) of the United Nations, the International Atomic Energy Agency (IAEA), and Codex Alimentarius have all approved the technology's safety. Food radiation has the potential to increase food's shelf life while preserving its organoleptic and secure qualities (Iqbal *et al*., 2013). Depending on the variety of the ingredients, gamma irradiation between 5.0 and 10.0 kGy usually meets expectations in removing microbial contamination and insects without changing the chemical or taste and flavor profile (Verma *et al*., 2015). However, the original microbial load and the type of microorganism present in the ingredients largely determine the amount of radiation needed to decontaminate (Balakrishnan *et al*., 2022). In general, many studies find that fungi could be killed with radiation doses that were much lower than those required to eliminate the microbial load (Prakash *et al*. 2011). There is a positive correlation between the amount of total aflatoxins that are reduced in all of the samples that were analyzed in the current study and the amount of gamma irradiation applied to the samples; however, the highest aflatoxin reduction percentage was attained at 20 kGy; it reaches 90% of total aflatoxin in frozen meat followed by 72.49%, 62.53%, 60.49%, 55.82%, 55.52%, 52.57%, 52%, 51.74%, 39.3%, 38.88% and 35.68% in mloha, minced meat, beef burger, beef luncheon, chicken luncheon, chicken frozen liver, chicken burger, kofta, Fesikh, beef sausage, and beef frozen liver respectively. While at 10 kGy; the reduction percentage reaches 85.82% of total mycotoxins in frozen meat followed by 53.43%, 50.98%, 49.55%, 44%, 40.74%, 34.6%, 34.37%, 33.7%, 28%, 27.42% and 27.02% in minced meat, beef burger, mloha, chicken luncheon, chicken burger, chicken frozen liver, beef luncheon, kofta, Fesikh, beef sausage, and beef frozen liver respectively (Tables 6, 7). These findings suggest that aflatoxins in food may be degraded by gamma irradiation to concentrations below the upper limit permitted. The present findings are consistent with Awad *et al*. (2019), who found that at 10 kGy, the maximum reduction percentage of mycotoxins was attained. It reaches 37.88% for total mycotoxins, 27.22% for AFB1, 40.12% for AFB2, 63.04% for AFG1, and 90.24% for AFG2. Furthermore, according to Ghanem *et al*. (2008), the greatest percentages of AFB1 degradation at 10 kGy dose were 58.6, 68.8, 84.6, 81.1, and 87.8% for the samples they looked at. While Vita *et al*. (2014) claimed that the maximum reduction was discovered at 15 kGy

and that it was 19.25%, 10.99%, 21.11%, 16.62%, and 23.9% for AFB1, AFB2, AFG1, and AFG2, respectively.

Given this, general and public education is required to inform the public of the financial and health risks presented by mycotoxins. People should be made aware of control measures like good ingredient selection, appropriate washing, farming practices, and cooking techniques for food commodities. To reduce the negative effects of mycotoxins in food, regulatory control, quick and effective analyses and detection, and proper product handling and storage should be promoted. If consumers are given accurate information about food radiation, they are more ready and willing to accept radiated foods. Numerous studies conducted around the globe revealed that consumers generally accepted radioactive foods (Maherani *et al*., 2016).

CONCLUSION

According to the findings of the current study, the majority of the products under examination were contaminated with one or more aflatoxin(s) of various types of mould, which are thought to be the primary cause of the deterioration of meat products, causing significant financial losses and posing a risk to the public's health by producing a wide range of mycotoxins. Preventing the development of fungi in the substrate is one of the most efficient ways to manage the issues brought on by aflatoxins.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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