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# Prevalence of Mould and Aflatoxin in Raw and Heat-Treated Meat Products

Mohamed A. Hussein[1], Ahmed E. Tharwat[1], Rana M.M. Ali[1*], Elham E. Abo-Almagd[2], Basma A. Fakhry[3]

[1]Food Control Department, Faculty of Veterinary Medicine, Zagazig University, 44511 Zagazig, Egypt.

[2]Department of Nutrition, Oncology center, Faculty of Medicine, Mansoura University, Egypt.

[3]Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig university, Egypt.

***Correspondence**
Corresponding author: Rana M.M. Ali
E-mail address: rana.vet880@gmail.com

## Abstract

From several stores and butchers in Mansoura city, Dakahlia Governorate, Egypt, 120 samples of sausage, beef burger, minced meat, luncheon, hot dog and canned meat were collected (20 each). The samples examined for detection of total mould count and identification if mould into genera and species in addition to quantification of aflatoxin B1, B2, G1 and G2. Mould detected in 100% of all examined raw meat products meanwhile, detected in 25%, 30% and 15% of examined luncheon, hotdog, and canned meat, respectively. Heat treated meat products significantly ($P<0.05$) contained lower mould count than raw meat products. Eight mould genera detected in all examined meat products with varying percentages in descending order *Aspergillus* > *Penicillium* > *Cladosporium* > *Sporotricum* > *Alternaria* > *Mucor* > *Fusarium* > *Curvularia* . The mean values of aflatoxin B1 were 0.78±0.21, 1.1±0.55, 1.54±0.40, 0.052±0.032, 2.21±0.87 and 1.88±0.41 µg/kg in sausage, beef burger, minced meat, luncheon, hot dog and canned meat, respectively. Minced meat significantly lower than other examined meat products in level of aflatoxin B1($P< 0.05$). Aflatoxin B2 and G2 not detected in all examined samples. The aflatoxin G1 detected in two samples of beef burger with a mean value 1.15±0.065 µg/kg and in one sample of canned meat 0.62 µg/kg. A food safety management system as hazard analysis and critical control points should be adopted by meat producers in order to protect human health.

KEYWORDS
Aflatoxin, *Aspergillus*, *Penicillium*, Meat products

## INTRODUCTION

Meat products are a rich source of concentrated nutrients since they include protein with a high digestibility score as well as vitamins, minerals, vital amino acids, and fatty acids that are believed to be important for healthy human growth in both young and adult populations. Moreover, provide clients with a source for fast, inexpensive, and nutritious meals, which they highly value for its flavor, affordability, and simplicity of preparation (Hussein *et al*., 2018). The Inadequate sanitary and hygienic conditions during handling, processing, and storage were the cause of meat product mold contamination. It is possible to enhance the amount of mold contamination in such items by adding flavoring agents of poor quality. Spices and other flavorings added to the meat product mix can significantly enhance the meat's mold contamination (Habashy *et al*., 2019). Mycotoxins are a wide and potent class of poisonous chemicals with adverse effects on both humans and animals (Bennett and Klich, 2003). They contaminate a wide range of foods and are secondary fungal compounds generated during mould development. Grains, rice, beans, coffee, wine, fruits, nuts, spices, eggs, and animal products are among the foods considered to be the riskiest. The issue is that, despite research efforts and mitigation measures, their occurrence is not entirely prevented. Carcinogenicity, teratogenicity, immunological toxicity, neurotoxicity, hepatotoxicity, nephrotoxicity, reproductive and developmental toxicity, indigestion, and other negative consequences have been seen in both people and animals (Pleadin *et al*., 2019). Food safety and hygiene can still be compromised by mycotoxins, viruses, and bacteria (De Ruyck *et al*., 2015). Although the FAO reported that cereals were 25% mycotoxin-contaminated in 1999, more current statistics reveal that contamination to be substantially greater (about 60–80%) (Eskola *et al*., 2019). Studies have shown that there are various variables, including the kind of mycotoxin and the analytical or reporting methods employed, that affect the worldwide mycotoxin prevalence in crops. In addition, the vast 2008–2017 research on various cereals and their derivatives conducted in almost a hundred nations demonstrated a high correlation between mycotoxin incidence and meteorological circumstances. Most samples (88% were positive for at least one mycotoxin) and their co-occurrence (64% of samples were positive for at least two mycotoxins) were found to contain mycotoxins (Gruber-Dorninger *et al*., 2019). According to evidence, there are three ways that meat products can become contaminated: (i) through contaminated raw materials like spices and other ingredients; (ii) through mycotoxin-producing molds found on the surface of dry-cured meat products; and (iii) through the spillover effect from farm animals exposed to contaminated feed (Asefa *et al*., 2011; Bertuzzi *et al*., 2013; Pleadin *et al*., 2013). Mycotoxins frequently linger in raw materials and finished goods and build up in the body of humans, leading to serious health problems that are brought on by eating contaminated food (Richard, 2007; Duarte *et al*., 2010). The current


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study was designed to evaluate the level of mould contamination in addition to determination of aflatoxin residues in raw and heat-treated meat products.

## MATERIALS AND METHODS

*Collection and preparation of samples*

One hundred twenty samples of sausage, beef burger, minced meat, luncheon, hot dog and canned meat (20 each) were chosen at random from several stores and butchers in Mansoura city, Dakahlia Governorate, Egypt. The samples collected from April through August 2022. In order to evaluate samples mycologically without undue delay as well as detection of aflatoxin, samples were immediately moved to the laboratory of Food Control Department, Faculty of Veterinary Medicine, Zagazig University, under completely aseptic conditions after being stored in sterile polyethylene bags and kept in an ice box. A total of 25 g from each sample were aseptically homogenized at 2500 rpm for two minutes in 225 ml of 0.1% sterile peptone water using a sterile homogenizer. As a result, homogenate indicates a dilution of 10-1, and decimal dilutions were subsequently performed (APHA, 2001).

*Estimation and Identification of mould count*

Total mold count was calculated by growing duplicate plates on several types of malt extract agar media (MEA) (Oxoid) and incubating them at 25°C for one to seven days. Every day throughout the incubation period, the plates were checked for the development of the star-shaped mold (APHA, 2001). Under aseptic circumstances, mold colonies were collected, then subcultured on MEA slopes and stored for further analysis. The identification of the mold colonies was done by meticulous observation and measurements of the colonies' macroscopic and microscopic features, which were then documented on data sheets (Pitt and Hocking, 2009). The uniformity of the surface growth, the pattern of folding (rugae), the clarity of the colony edge, and the presence of pigment on the colony's surface, its reversal, or dilating into the surrounding media are all things that may be observed during a macroscopical study. A magnifying hand lens was used to view the colony's front and rear sides. microscopical analysis A portion of the colony was quickly stained with a few drops of lactophenol cotton blue stain using mycological needles, then covered with a clean cover slide. The micromorphological characteristics of the head, vesicle, sterigmata, conidiophore, and conidia were evaluated on each slide using low power and high-power magnifying lenses.

*Determination of aflatoxin residues*

The standards of aflatoxin (B1, B2, G1 and G2) were obtained from Biocomma limited, China. The column capacity and recovery as shown in Table 1. The sample was divided into 100 grams, homogenized, and thoroughly mixed with 10 ml of 20% citric acid made by adding 200 ml of dichloromethane. The mixture was then shaken on a timer for 30 minutes. The mixture was separated, and the components that had been filtered were then vacuum-evaporated. Finally, the extracted material was given hexane. Solid-phase extraction (SPE) columns, Bond Elut C18 (500 mg, 3 ml, or 6 ml; Varian, Les Ulis, France), were used for cleanup. To remove lipids, the extracted materials were added to the gel on top of the column and eluted with hexane. A 1:3:6 ratio of hexane, ether, and acetonitrile was used to eliminate further contaminants (Herzallah, 2009). To acquire or recover aflatoxins, the column was eluted using an elution solution that was a combination of dichloromethane and acetone. Nitrogen evaporators (Turbo Vap® LV, Caliper) were used to evaporate the organic solvent until it was completely dry. Using a gradient approach with a flow rate of 1 ml/min at a temperature of 30°C, 20 microliters of the solution was injected into HPLC along with an isocratic mobile phase made up of deionized water, acetonitrile, and methanol (60:20:20 v/v/v). A reversed-phase column (Extend-C18, Zorbax column, 4.6 mm 250 mm, 5 m, Agilent Co.) was used for the separation. A fluorescence detector with wave lengths of 360 nm excitation and 440 nm emission was used to do the detection. The area under the curves, which was automatically extrapolated using ChemStation software, was used to extract and compute the quantity of residues in the samples.

*Statistical analysis*

The one-way ANOVA significant at $P < 0.05$ and Pearson correlation tests were used to evaluate the data.

## RESULTS

According to Table 2, mould detected in 100% of all examined

Table 1. Column capacity, recovery, and rate of standard deviation of aflatoxin B1, B2, G1 and G2

| Fraction | Addition level | Testing results (ng) | | | | | Recovery (%) |
|---|---|---|---|---|---|---|---|
| | | Rep.2 | Rep.2 | Rep.3 | Avg. | RSD (%) | |
| B1 | 77.256 ng | 76.2 | 79.5 | 75.4 | 77 | 2.8 | 99.7 |
| B2 | 16.488 ng | 143 | 13.8 | 14 | 14 | 1.8 | 84.9 |
| G1 | 82.224 ng | 75.9 | 77 | 79.1 | 77.3 | 2.1 | 94 |
| G2 | 24.408 ng | 20.3 | 19.3 | 20 | 19.9 | 2.6 | 81.5 |

Table 2. Prevalence and count of mould $log^{10}$CFU/g in examined raw and heat-treated meat products (N= 20 for each).

| | Raw meat products | | | Heat treated meat products | | |
|---|---|---|---|---|---|---|
| | Sausage | Beef burger | Minced meat | Luncheon | Hot dog | Canned meat |
| Prevalence | (20/20) 65% | (20/20) 85% | (20/20) 45% | (5/20) 25% | (6/20) 30% | (3/20) 15% |
| Minimum | 2.36 | 2.21 | 1.24 | 2.1 | 1.24 | 1.25 |
| Maximum | 4.21 | 5.2 | 3.33 | 2.44 | 2.54 | 2.65 |
| Mean±SD | $3.32±0.71^{a}$ | $3.50±0.91^{a}$ | $2.43±0.58^{b}$ | $1.91±0.62^{c}$ | $1.95±0.52^{c}$ | $1.42±0.65^{c}$ |

($^{a, b, c}$) different superscript letters in the same row indicate significant differences ($p < 0.05$).

raw meat products meanwhile, detected in 25%, 30% and 15% of examined luncheon, hotdog, and canned meat, respectively. Heat treated meat products significantly (P<0.05) contained lower mould count than raw meat products. Eight mould genera detected in all examined meat products with varying percentages in descending order *Aspergillus* > *Penicillium* > *Cladosporium* > *Sporotricum* > *Alternaria* > *Mucor* > *Fusarium* > *Curvularia* (Table 3). The recorded data in Table 4 declared that A.niger and A. flavus detected in 40% and 20%, 35% and 30%, 25% and 10% in sausage, beef burger, respectively. The A. fumigatus detected in 10%, 10%, 5% and 5% of examined sausage, beef burger, minced meat and luncheon, respectively. The A. ochraceus detected in 10% of examined sausage and beef burger while detected in 5% of examined luncheon, hot dog and canned meat. The mean values of aflatoxin B1 were 0.78±0.21, 1.1±0. 55, 1.54±0.40, 0.052±0.032, 2.21±0.87 and 1.88±0.41 µg/kg in sausage, beef burger, minced meat, luncheon, hot dog and canned meat, respectively. Minced meat significantly lower than other examined meat products in level of aflatoxin B1(P< 0.05). Aflatoxin B2 and G2 not detected in all examined samples. The aflatoxin G1 detected in two samples of beef burger with a mean value 1.15±0.065 µg/kg and in one sample of canned meat 0.62 µg/kg (Table 5).

## DISCUSSION

Mold contamination of food is a severe problem everywhere on the globe. Not only producing economic losses but also creating risks for both humans and animals. One of the indicative indicators of a product's hygienic status, which describes the environment, the state, and the circumstances surrounding the production process, is the presence of mold in the meat. In the current study, as recorded in Table 2 the total mold count of sausage (3.32±0.71 $\log_{10}$CFU/g) comparable mould counts 3.08 $\log_{10}$CFU/g (El Bayomi *et al*., 2021), 3.04±2.15 $\log_{10}$CFU/g (Abuzaid *et al*., 2020), 3.12±1.23 $\log_{10}$CFU/g (Hamad *et al*., 2021). Meanwhile, higher mould count in sausage 4.6±4.1 $\log_{10}$CFU/g (Abdel Gawaad and El Leboudi, 2005). The mean value of mould was 3.50±0.91 $\log_{10}$CFU/g in examined beef burger samples which slightly higher than 2.85 $\log_{10}$ CFU/g (El Bayomi *et al*., 2021), 2.87±1.79 $\log_{10}$ CFU/g (Hamad *et al*., 2021) and 2.84 $\log_{10}$ CFU/g (Maktabi *et al*., 2016). The mould count in minced meat was 2.43±0.58-$\log_{10}$ CFU/g which come in consistent with 2.24 $\log_{10}$ CFU/g (El Bayomi *et al*., 2021), 2.24±1.72 $\log_{10}$ CFU/g (Algammal *et al*., 2021) and 2.4±2.0 $\log_{10}$ CFU/g (Saad *et al*., 2015). Meanwhile, higher count 3.2±2.8 $\log_{10}$ CFU/g (Hassan *et al*., 2014). The mould count in luncheon and hot dog samples slightly lower than 2.27 $\log_{10}$ CFU/g (Ebraheem and Ghadam, 2015) and 2.74±1.3 $\log_{10}$ CFU/g (Hamad *et al*., 2021). The mould count in canned meat was lower than 3.28 $\log_{10}$ CFU/g in canned meat from Kingdom Saudi Arabia (Nasser, 2015).

The mould species isolated fromsausage, beef burger, minced meat, luncheon, hot dog and canned meat were nearly similar with those obtained in national studies (Abdel Gawaad and El Leboudi, 2005; Saad *et al*., 2015; Habashy *et al*., 2019; El Bayomi *et al*., 2021) and international studies (Nasser, 2015; Maktabi *et al*.,

Table 3. The number and proportion of identified mould genera in examined raw and heat-treated meat products (N= 20 for each).

| | Raw meat products | | | Heat treated meat products | | |
|---|---|---|---|---|---|---|
| | Sausage | Beef burger | Minced meat | Luncheon | Hot dog | Canned meat |
| *Aspergillus* | 17 (85%) | 18 (90%) | 11 (55%) | 3(15%) | 2 (10%) | 1 (5%) |
| *Penicillium* | 11 (55%) | 9 (45%) | 6 (30%) | 2 (10%) | 1 (5%) | - |
| *Cladosporium* | 8 (40%) | 9 (45%) | 3 (15%) | - | 2 (10%) | 2 (10%) |
| *Sporotricum* | 6 (30%) | 7 (35%) | 2 (10%) | - | - | 1 (5%) |
| *Alternaria* | 3 (15%) | 4 (20%) | - | 1 (5%) | 2 (10%) | 1 (15%) |
| *Mucor* | 2 (10%) | 3 (15%) | 1 (5%) | 1 (5%) | - | - |
| *Fusarium* | - | 2 (10%) | - | 1 (5%) | - | - |
| *Curvularia* | 2 (10%) | 1 (5%) | 1 (5%) | - | 1 (5%) | 1 (5%) |

Table 4. The number and proportion of identified *Aspergillus* species in examined raw and heat-treated meat products (n=20).

| Species | Raw meat products | | | Heat treated meat products | | |
|---|---|---|---|---|---|---|
| | Sausage | Beef burger | Minced meat | Luncheon | Hot dog | Canned meat |
| *A. niger* | 8 (40%) | 7 (35%) | 5 (25%) | 1 (5%) | - | - |
| *A. flavus* | 4 (20%) | 6 (30%) | 2 (10%) | - | 1(5%) | - |
| *A. fumigatus* | 2 (10%) | 2 (10%) | 1(5%) | 1(5%) | - | - |
| *A. ochraceus* | 2 (10%) | 2 (10%) | - | 1(5%) | 1(5%) | 1(5%) |
| *A. terreus* | 1 (5%) | - | - | - | - | - |
| *A.parasiticus* | - | 1 (5%) | 3(15%) | - | - | - |

Table 5. Aflatoxin residues (µg/kg) in examined raw and heat-treated meat products (n=5).

| | Raw meat products | | | Heat treated meat products | | |
|---|---|---|---|---|---|---|
| | Sausage | Beef burger | Minced meat | Luncheon | Hot dog | Canned meat |
| B1 | 1.14 -3.14<br>1.88±0.41$^{ab}$ | 1.25- 4.12<br>2.21±0.87$^{a}$ | 0.010-0.016<br>0.052±0.032$^{c}$ | 0.9-2.18<br>1.54±0.40$^{ab}$ | 0.98-2.14<br>1.1±0. 55$^{b}$ | 0.38-0.85<br>0.78±0.21$^{b}$ |
| B2 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| G1 | 0.95 | 1.11- 1.19<br>1.15±0.065 | <LOD | <LOD | <LOD | 0.62 |
| G2 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |

($^{a, b, c}$) different superscript letters in the same row indicate significant differences (p < 0.05). <LOD: below the limit od detection.

2016; Zadravec *et al*., 2020). The mould count Significantly, lower (P< 0.05) in heat-treated meat products than raw meat products come in consistency with fact that most molds are heat-sensitive and affected with heat treatments between 60-71°C (Breidt and Costilow, 2004). Meanwhile presence of mould in heat-treated product with a few percentages and counts may attributed to post processing contamination or and inadequately heat-treated as recommended. The sausage and beef burgers had the highest level of contamination because of the ingredients in both products, which are minced meat blended with different spices, binders, and extenders. These ingredients are typically imported from developing nations with tropical and subtropical climates, where hot temperatures, copious amounts of rain, and humidity frequently encourage fungal growth and facilitate the occurrence of mycotoxin (Pickova *et al*., 2020). There are multifactorial causes affect the level of meat product contamination during processing as grinding, mincing, cooking, chilling, and packing. Mold may readily get into the meat products through all of these procedures. Therefore, the presence of mold in meat products indicates that these items were prepared in unhygienic processing.

Aflatoxin accumulation in the liver after being consumed in contaminated food, even in little levels, and has a carcinogenic impact. Given that ingesting 28 mg of AFB1 over a lifetime can cause cancer, even extremely low concentration levels (1 ppb) would pose a serious risk to the public's health (Garner, 1992). The most toxic mycotoxin for both people and animals, aflatoxin B1 (AFB1), has been designated as a Group 1 human carcinogen (IARC, 2002). The studied samples varied significantly from one another (P< 0.05). Furthermore, the descending manner for AflatoxinB1 was arranged as follow beef burger > sausage > luncheon > hot dog > canned meat > minced meat. The aflatoxin B1 seems to be correlated with number of spices and additives that had previously been contaminated with aflatoxins may be the reason why the lowest amount of aflatoxin B1 was found in minced meat. Previous national studies declared the level of aflatoxin B1 as 10.4±5.1, 2.3±0.4 ppb in luncheon, basterma (Ismail *et al*., 213) and 7.23 ± 0.8, 5.63 ± 0.95, 4.88 ± 0.11, 2.03 ± 0.3 ppb luncheon, hot dog, corned beef, and minced meat, respectively (Algahtani *et al*., 2020). Furthermore, aflatoxin B1 detected globally at level 7-8 ppb in Italy from sausage (Iacumin *et al*., 2009), 1.9–6.3 ppb from dry-cured Iberian ham in Spain (Rodríguez *et al*., 2012), and up to 1.92 in traditional meat products from Croatia (Zadravec *et al*., 2020). All examined samples were below the maximum permissible limit for aflatoxin B1 (5 ppb) as maximum level established as regulation for European countries (EC, 2006). Regarding the aflatoxin G1level which come lower than 4.37 ± 0.63, 5.63 ± 0.2, 6.9 ± 0.63 and 5.53 ± 0.1 ppb in luncheon, hot dog, corned beef, and minced meat, respectively (Algahtani *et al*., 2020). Aflatoxin B2 and G2 were not detected in all examined samples on contrary detected as 5.20±0.69 and 3.35±0.49 in sausage, 5.57±0.72 and 3.84±0.58 ppb in luncheon (Shaltout *et al*., 2014).

## CONCLUSION

Meat products in this investigation were contaminated to different mould and aflatoxin B1 which indeed below the permissible limit but long-term consumption may lead to public health hazard. In order to maintain hygienic conditions during processing, preparation, and handling, a concentrated effort should be made. This should be avoided by using hygienic procedures while slaughtering of animals and preparation of meat, selection of species and additive, proper packaging and cooling of raw meat products, proper heat treatment of heat-treated products and training of meat factory workers.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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