Estimation Of Aflatoxin Residues In Some Meat Products

Wafaa F Ahmed*, Manal M El Mesallamy and Nahed M El Mokhtar*****
*Food Hygiene Dept., **, Microbiology Dept Zagazig Provincial Lab., and *** Mycology Dept.
Animal Health Institute, Egypt

ABSTRACT

A total of 90 samples of sausage, luncheon, and burger of beef origin (30 from each) were collected from Zagazig City markets for detection the total aflatoxin residues. The obtained results revealed that the mean levels of the total aflatoxin residues were 2.46 ± 0.660, 2.50 ± 0.554 and 1.80 ± 0.369 ppb in the examined sausage, luncheon, and burger samples respectively. The total aflatoxin levels exceeded the European permissible limits (4 ppb) in 4 (13.33%), 3 (10%) and 2 (6.66%) of the examined sausage, luncheon and burger samples respectively. Meanwhile, only 2 (6.66%) and 1 (3.33%) of the sausage and luncheon samples respectively contained total aflatoxin residues in levels above the Egyptian standard (2003) permissible limits (10 ppb). The frequency distributions of the total aflatoxin residues within the different meat product samples indicated relatively wide range of aflatoxin distribution in sausage and luncheon samples in comparing with those in burger. Upon the probable sources of aflatoxin residues, the hygienic storage of animal feed, animal feed ingredients and meat products are highly recommended to avoid fungal infection and subsequently mycotoxin residues. Furthermore, the choice of the good quality meat, spices and food additives are also recommended.

INTRODUCTION

Mycotoxins are toxic metabolites synthesized by some naturally occurring fungi under suitable physical, chemical and biological factors. High temperature, stress, humidity stress and insect damage of the product are major determining factors in mold infestation and toxin production. Mycotoxins contaminated food and feed supplies could increase the economic and health risks to humans and animals. The aflatoxins constitute a group of fungal metabolites that have varied toxic and carcinogenic properties, depending on dose and duration of exposure *(1)*.

Aflatoxin is one of the more important group of mycotoxins. It has a wide occurrence in different kind of materials, such as spices, cereals, oils, fruits, vegetables, milk, meat. Humans can be exposed to aflatoxins by the periodic consumption of contaminated food, contributing to an increase in nutritional deficiencies, immunosuppresion and hepatocellular carcinoma, exposure to aflatoxin is known to cause both chronic and acute hepatocellular injury. For example, in Kenya, acute aflatoxin poisoning results in liver failure and death in up to 40% of cases *(2)*. Furthermore, Aflatoxin may be regarded as a quadruple threat as a potent teratogen and mutagen *(3)*. Among the 18 different types of aflatoxins identified, the major members are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2) which are produced by *Aspergillus flavus* and/or *Aspergillus parasiticus*. Strains of *A. flavus* can vary from non-toxic to highly toxigenic and are more likely to produce AFB1 than AFG1. Strains of *A. parasiticus* generally have less variation in toxigenicity and produce AFB1 and varying amounts of AFB2, AFG1 and AFG2 *(4)*.

Meat products are important source of animal protein, the consumption of these products was highly developed in Egypt especially within youth and children. Although meat products constitute high quality, easily

especially within youth and children. Although meat products constitute high quality, easily prepared, cooked and good taste animal protein, yet it is exposed to different types of fungi *(5)*. Thus, these fungi may be a source of aflatoxin contamination as described previously. Moreover, the animal feed contaminated by *Aspergillus flavus* and *Aspergillus parasiticus* exposed to aflatoxin contamination. Thus; subsequently, animal meat and meat products may be suffered from aflatoxin residues *(6)*.

The aim of the present investigation was to estimate the total aflatoxin residues (AFB1+ AFB2 + AFG1 + AFG2) in locally produced sausage, luncheon, and burger samples from Sharkia Governorate markets.

MATERIAL AND METHODS

Collection of samples

A total of 90 samples of sausage, luncheon, and burger of beef origin (30 from each) were collected from Zagazig City markets. All samples were kept frozen at -4°C in polyethylene bages after collection till analysis.

Extraction of samples

Samples extraction was carried out as previously described method *(7)*. About 50 gm. from each meat product sample was blended till become homogenous and transferred into 500 ml wide- mouth glass-stopper Erlenmeyer flask. Ten ml of 20% citric acid solution were added and mixed thoroughly with glass stirring rod. After 5 minutes, stirred again and mixed with 20 gm diatomaceous earth. Then 200 ml. dichloromethane was added. Flask was shaked vigorously on the wrist action shaker for 30 minutes. The mixture was filtered through fast flow paper into 300 ml Erlenmeyer flask containing 10gm of anhydrous sodium sulfate (filter top was closed and compressed entire filter against funnel to obtain the maximum filtrate volume). Gently swirl flask intermittently about 2 minutes and re-filtrated through medium flow paper into 250 ml graduated cylinder and the volume was recorded. The filtrate was evaporated in 500 ml round flask under vacuum to near dryness and saved for column chromatography.

Column chromatography

The extracted samples were cleaned up and purified using silica solution through column *(8)*. Column was filled half full with dichloromethane and 2.0 gm. silica gel was added. Add 3-4 ml dichloromethane and slurry silica with stainless steel rod. Drain dichloromethane to still silica and rinse silica off column side with dichloromethane. two gm. anhydrous sodium sulphate were added to supernatant solvent above silica gel to cap column and drain excess dichloromethane to about 1.0ml above column packing. Concentrated filtrate was re-dissolved in about 25ml dichloromethane, add to column and drain entire solution through column with gravity. Column was washed with 25 ml toluene – acetic acid (9 + 1) then with 25 ml hexane and 25 ml hexane – ether – acetonitrile (6 + 3 + 1), discard washes. Aflatoxin was eluted with 40 ml dichloromethane – acetone (4 +1) and evaporated the elute to near dryness on steam bath then save for Thin Layer Chromatography (TLC) analysis was carried out in Animal Health Research Institute, El-Dokki, Geza.

Detection of aflatoxin residues

The extracted purified samples were analyzed for detection of aflatoxins using Thin Layer Chromatography (TLC) according to the perviously described technique *(9)*.

Statistical analysis

Statistical analysis of data was carried out *(10)*.

RESULTS AND DISCUSSION

Table 1. Concentrations of the total aflatoxin residues (ppb) in the examined meat product samples (n= 30 for each)

	Sausage n=30	Luncheon n=30	Burger n=30
Maximum	16.0	15.0	9.0
Minimum	N.D.	N.D.	N.D.
Mean ±SE	2.46 ±0.660[a]	2.50 ±0.554 [a]	1.80 ±0.369 [b]

N.B.: The difference between letters means the variation between the values of the aflatoxin residues in the meat product is significant at level ($p \leq 0.05$).

Table 2. The comparison between the total aflatoxin levels in the examined meat products with their permissible limits. (n= 30 for each)

Samples	European limit (4 ppb) *(11)*				Egyptian limit (10 ppb) *(12)*			
	Within P.L.		Over P.L.		Within P.L.		Over P.L.	
	No.	%	No.	%	No.	%	No.	%
Sausage	26	86.66	4	13.33	28	93.33	2	6.66
Luncheon	27	90	3	10	29	96.66	1	3.33
Burger	28	93.3	2	6.66	30	100	0.0	0.0

The obtained results of the total aflatoxin levels in the examined samples are showed in Table 1. These levels nearly similar to those recorded in the ostrich meat and meat products in Egypt *(13)* which detected total aflatoxins residues in levels ranged between 1.4 to 2.8 ppb. Moreover, our estimations located within the wide range of the recorded total aflatoxin residues in basterma samples in Egypt (2.5 – 74 ppb) *(14)*, and in fresh meat samples in Jordan (0.15- 6.36 ppb) *(15)*. On the other hand, aflatoxin residues were not detected in all the examined samples of pork meat and meat products in previous study in Romania *(16)*. The statistical analysis revealed no significant variations of the total aflatoxin levels between sausage and luncheon samples; while, these levels in burger samples recorded significant lower values than mentioned in the two other meat products. These variations may be explained by the natures and quantity of the meat product additives and spices, because these materials usually contained higher aflatoxin residues than the crude meat itself *(14)*.

Table 2 exhibited that total aflatoxin levels exceeded the European permissible limits (4 ppb) in 4 (13.33%), 3 (10%) and 2 (6.66%) of the examined sausage, luncheon and burger samples respectively. Meanwhile, only 2 (6.66%) and 1 (3.33%) of the sausage and luncheon samples respectively contained total aflatoxin residues in levels above the Egyptian permissible limits (10 ppb). This result nearly coincided with those detected in the previous Egyptian study which recorded aflatoxin residues in levels exceeded the European permissible limits in 16.66% of the examined ostrich meat and liver samples *(13)*. These obtained results indicate aflatoxin safety levels in the most examined meat products according to the Egyptian standard.

Table 3. Frequency distribution of the total aflatoxin residues within the different meat product samples (n= 30 for each)

Frequency (ppb)	Sausage		Luncheon		Burger	
	No.	%	No.	%	No.	%
< 1.0	9	30	8	26.66	11	36.66
1.0 - <5.0	17	56.66	19	63.33	17	56.66
5.0 - < 10.0	2	6.66	2	6.66	2	6.66
10.0 - < 15.0	1	3.33	0.0	0.0	0.0	0.0
15.0 - < 20.0	1	3.33	1	3.33	0.0	0.0
Total	30	100	30	100	30	100

The frequency distributions of the total aflatoxin residues within the different meat product samples (Table 3) indicated relatively wide range of aflatoxin distribution in sausage and luncheon samples in comparing with those in burger. This result may be explained as previously mentioned by variations of quantity and natures of the meat product additive and spices between the examined meat product types.

From the obtained results, we could be concluded that the aflatoxin residues were detected in considerable levels in the examined meat products, although only few samples exceeded the aflatoxin permissible limits. Animal feed is the main source of the mycotoxin residues in alive animal body. Aflatoxin is the most predominant mycotoxin in the animal feed among the other mycotoxin types *(17)* and subsequently accumulated in animal tissues. Also, the meat products may exposed to fungal infection as recorded in previous study detected toxogenic strain of *Aspergillus flavus* in luncheon samples which is able to produce aflatoxin during storage and before selling *(18)*. Moreover, the spices and food additives are the important source of the aflatoxin residues as previously mentioned *(14, 18)*. Therefore, upon the probable sources of aflatoxin residues, the hygienic storage of animal feed, animal feed ingredients, and meat products are highly recommended to avoid fungal infection and subsequently mycotoxin residues. Furthermore, the choice of the good quality meat, spices and food additives are also recommended.

REFERENCES

1.Agag BI (2004): mycotoxins in foods and feeds 1-aflatoxins. Ass. Univ. Bull. Environ. Res. Vol. 7 No. 1.

2.CDC "Centrals for disease Control and Preventions" (2012): Understanding Chemical Exposures- Aflatoxin.

3.Ueno Y and Yeno I(1978): Toxicology and biochemistry of mycotoxins. In: Toxicology, biochemistry and pathology of mycotoxins. Ed. Urocguchi K. and Yamazaki M. Kodaansh Ltd. Tokyo, Japan.

4.Coppock WR and Christian RG (2007): Aflatoxins, In: Veterinary Toxicology – Basic and Clinical Principles, R. C. Gupta; pp. 939-950, Academic Press, ISBN 0123704677, San Diego.

5.Amal A. Mohamed and Nemmat A. Hussein (2004): Proteolytic and lipolytic activity of fungi isolated from luncheon meat and poultry in Assiut city. Assiut Vet Med. J. Vol. 50 No. 100.

6.Edrington T S, Harvey R B and Kubena L F (1995): Toxic effect of aflatoxin B_1 and ochratoxin A alone and in combination on chick embryos. Bull. Environ. Contam. Toxicol. 54: 331-336.

7.Stubblefield R D and Shotwell (1981): Determination of aflatoxin in animal tissues. J. Assoc. Off. Anal. Chem. 64 (4): 964-968.

8."AOAC" Offacial Methods Of Analysis 14th ed (1984): Natural poisons. Aflatoxins. Chapter 26, pp 477-494.

9.Leitao J, De saint G B, Bailly J R and Paillas C (1988): Quantitation of aflatoxins from various strain of Aspergillus in foodstuffs. J. Chromatogr. 435: 229-234.

10.Petric A and Watson P (1999): Statistics for veterinary animal science. 1st Ed., pp. 90-99. The Blackwell science Ltd, United Kingdom.

11.EU Commission Regulation "EC" (2003): No 2174/2003 of 12 December 2003 amending Regulation (EC) No 466/2001 as regards aflatoxins. Official J. European Union L 326 12-15.

12.Ministry of Agriculture Egyptian Standard (2003): UDC 615.91. Maximum Limits for Mycotoxin. In Foods Part I: Aflatoxin.

13.Nabela I Elsharkawy, Abou Elmagd MM and Salah-El-Dein WM (2006): Assessment of aflatoxin in ostrich flesh and its products. Zagazig Vet. Journal Vol. 34 No. (2) 140-147.

14.Refai MK, Niazi ZM, Aziz NH and Khafaga NE (2003): Incidence of aflatoxin B1 in the Egyptian cured meat basterma and control by gamma-irradiation. Nahrung. 2003 Dec;47(6):377-82

15.Herzallah SM (2009): Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. Food Chemestry, Volume 114, Issue 3, 1 pp 1141–1146.

16.Ilie L, Sayu C, Carmen Petcu L and Tudor F Furnaris (2007): Assessment Of Some Mycotoxins in meat and meat products. lucrări stiinłifice medicină veterinară vol. xl, timisoara. 418- 421.

17.Kocasari FS, Mor F, Ogu MN and Oguz FK (2012): Occurrence of mycotoxins in feed samples in Burdur Province, Turkey. Environ Monit Assess. Vol.4. [Epub ahead of print].

18.Ismail MA and Zaky Z M (1999): Evaluation of the mycological status of luncheon meat with special reference to aflatoxigenic moulds and aflatoxin residues. Mycopathologia. 146(3):147-54.

الملخص العربي

تقدير بقايا الأفلاتوكسين في بعض منتجات اللحوم

وفاء فتح الله أحمد*، منال محمد السيد، ناهد محمد المختار محمد*****

قسم صحة الأغذية*- قسم الميكروبيولوجي**- معمل الزقازيق الفرعي- ، قسم الفطريات***

معهد بحوث صحة الحيوان

أجريت هذه الدراسة لتقييم مستوى بقايا الأفلاتوكسين الكلي في بعض منتجات اللحوم و هي السجق، اللانشون والبيرجر ذات الأصل البقري، تم تجميع عدد ٩٠ عينة من أسواق مدينة الزقازيق (٣٠ من كل نوع)، و قد أسفرت النتائج عن الآتي، كان متوسط متبقيات الأفلاتوكسين الكلي ٥,٤٦، ٢,٥٠ و ١,٨٠ جزء في البليون في المنتجات المذكورة سابقا على التوالي. الدراسة الإحصائية أثبتت عدم وجود فروق معنوية بين بقايا الأفلاتوكسين بين كلا من السجق و اللانشون في حين كانت تلك البقايا أقل بشكل معنوي في البيرجر مقارنة بها في المنتجين السابقين، و قد يعزى ذلك لاختلاف نسب التوابل و الإضافات الغذائية من منتج لآخر و هي تحتوي عادة على نسب مرتفعة من بقايا الأفلاتوكسين أكثر من اللحم الخام.

فيما يخص المقارنة بين مستويات الأفلاتوكسين بالحدود القصوى المسموح بها، كانت تركيزات الأفلاتوكسين الكلي أعلى من الحدود الأوروبية المسموح بها (٤ جزء في البليون) في ٤ (١٣,٣٣%)، ٣ (١٠%) ، ٢ (٦,٦٦%) من عينات السجق البقري، اللانشون البقري و البيرجر البقري على التوالي. أما عن المقارنة بالحدود المصرية (١٠ جزء في البليون) فقد كانت تركيزات الأفلاتوكسين أعلى من تلك الحدود في ٢ (٦,٦٦%) ، ١ (٣,٣٣%) من عينات السجق و اللانشون على التوالي. و قد تمت مناقشة النتائج و اقتراح التوصيات المناسبة.