http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (5): 611-613, 2001

Mycoflora and Natural Occurrence of Mycotoxins in Some Meat Products and Livers of Poultry and Imported Bulls

[1]A.-L.E. Mahmoud, [2]A.M. Sayed and [2]A.A. Abou El-Alla
[1]Botany Department, Faculty of Science, Assiut University, Assiut 71516 , Egypt
[2]Animal Health Research Institute, Assiut Regional Laboratory, Assiut, Egypt

Abstract: Mycological analysis of local meat products (luncheon and minced meat) and livers of poultry and imported bulls resulted in isolation of 29 fungal species related to 10 genera. The average total counts of fungi per gram fresh weight ranged from 2680 in luncheon to 7460 in livers of poultry. *Aspergillus* was the most prevalent genus followed by *Penicillium* where they were isolated from all the examined substrates. Many of the isolated fungi might have mycotoxin-producing potential. Results of mycotoxins analysis revealed that, 45% of the examined samples were positive. Aflatoxins (B_1, B_2, G_1 and G_2), ochratoxin A, citrinin and sterigmatocystin were detected. Samples of livers from imported bulls contained the highest levels of aflatoxins and ochratoxin A (54 and 145 μg/kg, respectively). The majority of the remaining mycotoxin contaminated samples contained a high level which was far above the acceptable ones. The hazardous effects of these natural pollutants were discussed.

Key workds: Mycolfora, mycotoxins, livers, poultry, meat products.

Introduction

Contamination of meat products with moulds, leads to great economic loss, besides it constitutes a major public healt hazard. The contamination of meat products by species of *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* and *Cladosporium* were reported by many investigators (Zohri, 1990; Zaki *et al.*, 1995 and Nagat, 1997).

Mycotoxins, as secondary metabolites produced by many strains of moulds in different food and food products are highly toxic, non immunogenic, potent carcinogens and cause a potential hazard to human health (Bahgat, 1999). Humans are exposed to mycotoxins directly by consuming contaminated commodities or indirectly by consuming animal products or organs have ingested mycotoxins contaminated feeds (Hsieh, 1981).

Therefore the present study was designed to study the fungal contamination and assay for the natural occurrence of several mycotoxins in most popular meat products (Luncheon and minced meat) and edible organs (Livers of poultry and imported bulls).

Materials and Methods

Samples: Ten random samples of each of luncheon, minced meat, livers of poultry and livers of imported bulls were collected from different localities in Assiut City, Upper Egypt. Each sample (200 gm) was put in sterilized polythene bag. Samples were transferred immediately to the laboratory and each sample was divided into 2 parts. One part was kept in a deep freezer (-20°C) until mycotoxin analysis while the other part was used for mycological analysis.

Mycological analysis: This was made by using the dilution-plate method as described by Christensen (1963). A desired final dilution which supports a total of about 20-40 colonies per plate was used.

A modified glucose-Czapek's agar medium which contained: $NaNO_3$, 3: KH_2PO_4 1; $MgSO_4$, 0.5; KCl, 0.5; $FeSO_4$, 0.01; glucose, 10 and agar, 15 (gm/L of distilled water) was employed in this study. Rose bengal was used as a bacteriostatic agent (Smith and Dawson, 1944).

One ml of the desired dilution was transferred aseptically into sterile Petri-dish and 20-25 ml of the medium; cooled to just the solidification; were added. Three replicates of each sample were prepared. The dishes were incubated at 28°C for 7 to 10 days during which the growing colonies were identified and counted. The identification of fungi was according to De Vries (1952); Domsch and Gams (1972); Raper and Fennel (1965).

Mycotoxin analysis: 25 gm of each sample were homogenized with 100 ml of chloroform for 5 min. in a high speed blender. Extraction was repeated three times. The combined chloroform extract was washed by distilled water, dried over anhydrous sodium sulphate, filtered and concentrated to near dryness on a rotary evaporator. The residue was diluted with chloroform to one ml. The chloroform solution was analyzed for the presence of aflatoxins, ochratoxins, citrinin, sterigmatocystin, zearalenone T_2-Toxin and patulin using thin-layer chromatographic procedures (Gimeno, 1979).

The aflatoxin content was analyzed and confirmed using trifluoroacetic acid derivative formation (A.O.A.C., 1984). Citrinin and ochratoxin A were quantatively determined according to Scott *et al.* (1972) and Nesheim *et al.* (1973), respectively while sterigmatocystin quantity was determined by the method of Schroeder and Kalton (1975).

Results and Discussion

Mycological analysis of local meat products (Luncheon and minced meat) and livers of poultry and imported bulls resulted in isolation of 29 fungal species related to 10 genera (Table 1). The average total counts of fungi (per gm fresh weight) were 2680, 7340, 7460 and 4760 in luncheon, minced meat, livers of poultry and imported bulls, respectively. *Aspergillus* was the most prevalent genus followed by *Penicillium* where they were isolated from all the examined substrates. The remaining isolated genera were *Alternaria*, *Cladosporium*, *Fusarium*, *Mucor* and others (Table 1). Most of these genera were previously isolated but in different frequencies from chicken, meat and meat products in Egypt (Hefnawy, 1980; Hegazi *et al.*, 1992; Zaki *et al.*, 1995 and Nagat, 1997).

From *Aspergillus*, 13 species were identified of which *A. flavus* was the most common. This fungal specie was emerged in high occurrence from minced meat and livers of imported bulls, while it was moderately occurred in luncheon and livers of poultry. *A. terreus* and *A. niger* were isolated in high frequencies from minced meat and livers of poultry, while *A. ochraceous* and *A. parasiticus* were isolated from 30% of

Table 1: Average total counts, number of cases of isolation (out of 10 samples) and occurrence remarks of fungal genera and species isolated from luncheon, minced meat and livers of poultry and imported bulls

Fungal genera and Species	Luncheon			Minced meat			Livers of poultry			Livers of cattle		
	ATC	N.C.I	OR	ATC	N.C.I	OR	ATC	N.C.I	OR	ATC	N.C.I	OR
Alternaria alternata	100	2	L	140.0	2	L	180	2	L	-	-	-
Aspergillus	1820	6	H	5880.0	10	H	6300	7	H	4400	10	H
A. condidus	-	-	-	-	-	-	60	3	L	-	-	-
A. flavipes	-	-	-	40.0	1	R	160	2	L	-	-	-
A. flavus	740	4	M	1740.0	8	H	420	4	M	3180	9	H
A. fumigatus	-	-	-	380.0	4	M	440	4	M	380	3	L
A. nidulans	140	1	R	-	-	-	20	1	R	-	-	-
A. niger	220	3	L	N.980	6	H	940	6	H	240	2	L
A. ochraceus	360	3	L	Oc.260	1	R	140	1	R	-	-	-
A. oryzae	-	-	-	260.0	1	R	-	-	-	-	-	-
A. parasticus	-	-	-	-	-	-	-	-	-	200	3	L
A. sydowi	-	-	-	-	-	-	60	1	R	400	5	M
A. tamarii	-	-	-	-	-	-	260	3	L	-	-	-
A. terreus	220	2	L	1980.0	8	H	3480	7	H	-	-	-
A.versicolor	140	1	R	300.0	3	L	300	3	L	-	-	-
Cladosporium	80	1	R	440.0	5	M	400	3	L	-	-	-
C. cladosporioides	80	1	R	340.0	4	M	400	3	L	-	-	-
C. herbarum	-	-	-	100.0	1	R	-	-	-	-	-	-
Cochiolubolus spicifer	40	1	R	-	-	-	-	-	-	-	-	-
Drechslera spicifera	-	-	-	40.0	1	R	-	-	-	-	-	-
Fusarium	80	1	R	380.0	4	M	260	4	M	40	1	R
F. oxysporum	-	-	-	180.0	2	L	-	-	-	-	-	-
F.verticillioides	80	1	R	200.0	2	L	260	4	M	40	1	R
Mucor	40	2	L	180.0	2	L	-	-	-	-	-	-
M. circinilloides	-	-	-	80.0	1	R	-	-	-	-	-	-
M. hiemalis	40	2	L	100.0	1	R	-	-	-	-	-	-
Neurospora crassa	100	2	L	-	-	-	-	-	-	40	-	-
Penicillium	280	3	L	280.0	4	M	260	3	L	280	3	L
P. chrysogenum	60	1	R	160.0	2	L	180	2	L	-	-	-
P. corylophilum	40	1	R	-	-	-	-	-	-	-	-	-
P. funiculosum	-	-	-	-	-	-	80	1	R	-	-	-
P. viridicatum	180	2	L	120.0	2	L	-	-	-	280	3	L
Scopulariopsis	140	2	L	-	-	-	60	1	R	-	-	-
S. brevicaulis	140	2	L	-	-	-	-	-	-	-	-	-
S. koningii	-	-	-	-	-	-	60	1	R	-	-	
Gross total count	2680			7340.0			7460			4760		

ATC: Average total count (per gm fresh weight) M: Moderate occurrence, from 4 to 5 cases. N.C.L.: Number of cases of isolation
L: Low occurrence from 2-3 cases OR: Occurrence remarks R: Rare occurrence, one case only.
H: High occurrence, from 6 to 10 cases.

Table 2: Natural occurrence of mycotoxins in luncheon, minced meat, livers of poultry and imported bulls

Types of samples	Positive samples out of 10	Mycotoxin detected	Mycotoxin Concentration (µg/kg)	Mean concentration * (µg/kg)
Luncheon	4	Aflatoxins (B_1,B_2,G_1 and G_2)	11,22,27	100
		Ochratoxin A	95,105	20
Minced meat	4	Aflatoxins (B_1,B_2,G_1 and G_2)	18,22,29	23
		Citrinin	75,93,110,135	103
		Sterigmatocystin	115	115
Livers of poultry	5	Aflatoxins (B_1,B_2,G_1 and G_2)	29,43	36
		Citrinin	65,77,125,165	108
Livers of imported bulls	5	Aflatoxins (B_1,B_2,G_1 and G_2)	26,38,65,80	54
		Ochratoxin A	145	145

*Each value represents the mean of positive samples

luncheon and livers of imported bulls, respectively (Table 1), *Penicillium* incidence ranged from moderate in minced meat to low in luncheon, livers of poultry and livers of imported bulls. 4 *Penicillium* species were identified, of which *P. viridicatum* was isolated from 30% of livers of imported bulls and 20% of both luncheon and minced meat (Table 1). Nearly similar results were reported by Yassien *et al.* (1990); Salem (1991); and Zaki *et al.* (1995). In a similar study, *Aspergillus* and *Penicillium* were recorded as the predominant genera in minced meat, luncheon and pastirma (Abdel-Rahman *et al.*, 1984).

Regarding the results of mycological analysis of the examined samples (Table 1), many of the isolated fungi may have mycotoxin-producing potential. Therefore, the presence of mycotoxins in these samples could be expected. Mycotoxins can enter the food supply by direct contamination resulting from mould growth on the food, or indirectly through the use of contaminated ingredients in processed foods or by feeding mouldy feeds to food producing animals. In previous study, poultry feed ingredients were found to contain high levels of aflatoxins and Zearalenone (Mahmoud, 1993). Indirect contamination of food may be a problem in some area of the world where food is more highly processed (Bullerman, 1979; Bullerman *et al.*, 1984; Zohri, 1990 and Bahagt, 1999).

In the present study, 4 types of mycotoxins were detected of which aflatoxins were predominant and liver samples had the highest percentage of contamination (Table 2). Aflatoxins (B_1, B_2, G_1 and G_2) were detected in 4 samples of livers from

imported bulls with a mean concentration of 54 µg/kg. In addition to aflatoxins, ochratoxin A was found in one sample with concentration of 145 µg/kg. Mycological analysis of these samples showed that 90% and 30% of them were contaminated with *A. flavus* and *A. parasiticus*, respectively. Similar findings were reported by Bullerman (1979) and Refai (1988). Bullerman *et al.* (1984) reported that the liver is the critical animal tissue most likely to contain alfatoxin residues. On the other hand, Zaki *et al.* (1993) did not detect aflatoxins in liver, kidney and muscles of baldy bulls. This variation in results may be due to the fact that the level of aflatoxins contamination in feedstuffs is important factor influencing the tissue alfatoxin residues.

Citrinin was detected in 4 samples of both minced meat and livers of poultry with mean concentration of 103 and 108 µg/kg, respectively. It is worth to mention that, in the citrinin contaminated samples, aflatoxins were also detected in 3 and 2 samples of minced meat and livers of poultry with mean concentration of 23 and 36 µg/kg, respectively while sterigmatocystin was detected in one minced meat sample, with concentration of 115 µg/kg (Table 2). These results are in agreement with those of Hegazi *et al.* (1992) who found that 33% of minced meat samples contained aflatoxins. In a similar study, Bullerman (1979) observed that aflatoxins were mainly accumulated in the liver of poultry, duckling and turkey and could cause liver damage. No literature is available about the contamination of minced meat and livers of poultry with citrinin. We believe that this is the first report in this respect. The high level of citrinin in minced meat and livers of poultry may be attributed to the high occurrence of *A. terreus* on these substrates (Table 1).

40% of luncheon samples were contaminated with aflatoxins and ochratoxin A with mean concentration of 20 and 100 µg/kg, respectively. Mixed contamination was detected in one sample. Such results agreed with those recorded by Zohri (1990) and Zaki *et al.* (1995). Mycotoxin contaminations of luncheon may be originated either from the animal tissues previously fed on mycotoxins contaminated feed, or due to using mycotoxins contaminated ingredients e.g. cereals and spices (Zaki *et al.*, 1995).

The results of the present study have shown that the most examined samples were relatively highly contaminated by fungi and mycotoxins. The majority of mycotoxins contaminated samples contained a high levels which far above the acceptable ones (Bullerman, 1979 and Bahagt, 1999). Thus efforts have to be made to prevent mould growth and mycotoxin production along the entire food chain, from field to table. Another very important point in meat plants before mass slaughtering (especially in cases of imported bulls), representative numbers of apparently healthy animals must be analysed for occurrence of mycotoxins residues which if found by levels more than 20 µg/kg, slaughter should be delayed and the feeding of animals on mycotoxins free diets for 3-4 weeks as sufficient with holding period to clear the muscles and organs from toxins (Kerogh *et al.*, 1976).

References

A.O.A.C., 1984. Natural poisons. Official Methods of Analysis of the Association of Official Analytical Chemists, 447-484.

Abd El-Rahman H., H. Youssef and Hefnawy, 1984. Mycological quality of meat products in Egypt. Assiut Vet. Med. J., 12: 154-159.

Bahat M.E., 1999. Mycotoxins. A potential universal everlasting threat: "Consumer's perspective" 5th Sci. Cong., Egyptian Society for Cattle Diseases 28-30 Nov., page, 22-35.

Bullerman L.B., 1979. Significance of mycotoxins to food safety and human health. J. Food Prot., 42: 65-68.

Bullerman L.B., L. Schroeder and Kun-Young Park, 1984. Formation and control of mycotoxins in food. J. Food Prot., 47: 637-646.

Christensen C.M., 1963. Influence of small differences in moisture content upon the invasion of hard red winter wheat by *Aspergillus restrictus* and *A. repens*. Cereal Chem., 40: 385-390.

De Vries G.A., 1952. Contribution to the knowledge of the genus Cladosporium Link ex. Fr. Vitgevrij and Drukkerij. Hollandia Press Baarn.

Domsch K.H. and W. Gams, 1972. Fungi in agricultural soils. Published by Longman.

Gimeno A., 1979. Thin layer chromatographic determinaiton of aflatoxins, ochratoxins, sterigmatocystin, zearalenone, citrinin, T_2-toxin, diacetoxyscirpenol, penicilic acid, patulin and penitrem A.J. Assoc. Off. Analy. Chem., 62: 579-585.

Ferial El-Far S.M., A.M. Edreis and N.H. Aziz, 1992. Studies of Fungal and aflatoxins contamination of meat, meat products and food additives. Vet. Med. J., Giza, 40: 31-36.

Hseih P.H., 1981. Metabolism and transmission of mycotoxins. Proc. of the Int. workshop and symp. On mycotoxins, Sept. pp. 151-165. Nat. Res. Center, Dokki, Cairo, Egypt.

Kerogh P., F. Elling, B. Hald, A.E. Larsen, E.B. Lillehoj, A. Madsen and H.P. Movtensen, 1976. Time dependent disappearance of ochratoxin A residues in tissues of bacon pig. Toxicology, 6: 235-242.

Mahmoud A.-L.E., 1993. Toxigenic fungi and mycotoxin content in poultry feedstuff ingredients. J. Basic Microbiol., 33: 101-104.

Nagat A.S., 1997. Incidence of mycotoxins and mycotoxin-producing moulds in some Egyptian dairy products. 4th Sci. Cong., Egyptian Society for Cattle diseases, 7-9 Dec., p: 74-80.

Nesheim S., N.F. Hardin, Jr. O.J. Francis and W.S. Langham, 1973. Analysis of ochratoxins A and B and their esters in barley, using partition and thin layer chromatgraph, I-Development of the method. J. Assoc. Off. And anal. Chem., 56: 817-821.

Raper K.B. and D.I. Fennel, 1965. The genus Aspergillus. Williams and Wilkins, Baltimore, USA.

Refai M., 1988. Aflatoxins and aflatoxicosis. J. Egypt. Vet. Med. Giza, 48, No. 1, 1-19.

Salem R.M.T., 1991. Toxin producing fungi in imported frozen meat. M.V. Sc. Thesis, Fac. Med. Cairo Univ., Egypt.

Schroeder, H.W. and W.H. Kalton, 1975. Production of sterigmatosystin by some species of the genus *Aspergillus* and its toxicity to chicken embryos. Appl/Microbiol., 30: 583-585.

Scott P.M., W. V. Walbeek, B.P.C. Kennedy and D. Anyeti, 1972. Mycotoxins (ochratoxin A, citrinin and Sterigmatocystin) and toxigenic fungi in grains and other agricultural products. J. Agr. Food Chem., 20: 1103-1109.

Smith N.R. and V.T. Dawson, 1944. The bacteriostatic action of rose bengal in media used for the plate count of soil fungi. Soil Sci., 58: 467-471.

Yassien N., N. Mansour, E. El-Daley and A. Darweish, 1990. Contamination of slaughtered camels, cattle and its surroundings with mould in Cairo abbatoirs. Alex. Vet. Sci.

Zaki Z.M., M.A. Ismail and R.S. Refaie, 1995. Aspergillus flavus and aflatoxins residues in luncheon meat. Assiut Vet. Med. J. Vol. 33 No. 66: 114-117.

Zaki Z.M., A.A. Shaaban, A.A. Shehata and M.M. Saad, 1993. Aflatoxin residues in secretions, excretions and edible tissues of dairy and meat producing cattle. 2nd Sci. Cong. Egyptian Society for cattle Diseases, 5-7 Dec., Assiut, Egypt, p: 24-33.

Zohri A.A., 1990. Mycoflora and mycotoxins in some meat products. Ph.D. Thesis, Botany Dept., Fac. of Science, Assiut Unioversity, Assiut, Egypt.