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SOME STUDIES ON THE AFLATOXIN-PRODUCING ASPERGILLII IN MEAT-COLD STORES

(With 3 Tables and 3 Figures)

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بعض الدراسات عن عترات الأسبرجلس المفرزة لسموم الافلاتوكسينات فى ثلاجات حفظ اللّحوم

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يعتبر تلوث اللحوم ومنتجاتها بالفطريات من أهم المشاكل التي تهدد الصحة العامة للمستهلك حيث أن لها القدرة على إفراز السموم الفطرية والتي تعد الافلاتوكسينات من أهمها ،هذا بالإضافة إلى الدور الرئيسي الذي تلعبه الفطريات في فساد اللحوم ومنتجاتها والذي يؤدى إلى خسائر اقتصادية كبيرة بسبب إعدام تلك اللحوم أو منتجاتها. ولهذا اتجهت هذه الدراسة إلى الفحص الميكولوجى لعدد ١٠٠ مسحة من أرضية وأسطح وجدران ثلاجات حفظ اللحوم وكذلك الهواء واللحوم بداخلها بمدينة الإسماعيلية. وقد أمكن عزل ١٢ نوع من الفطريات معظمها ينتمي إلى الاسبرجلس، الكلادوسبوريوم، البنيسليوم، بنسبة (٣٤%)، (٩ر١٨٪) ، (٢ر١٦%) على التوالي. وقد تم تصنيف عدد ٩ أنواع من جنس الاسبرجلس وكان معظمها ينتمى الى أنواع الاسبرجلس فلافس لنك (٢٨%)، فلافس فاركولمنارس (٢٥%)،النيجر (١ر١٧%) ، النيديولنز (١١%)،الباراستيكس (٢ر٩%) . لقد وجدنا ٥٢(٧ر٤٧%)عترة سامة من بين ١٠٩ عترة من مجموعة الاسبرجلس فلافس المعزولة من جميع العينات المختبرة وقد تم التعرف على الافلاتوكسينات المفرزة بواسطة هذه العترات السامة وكانت بالمعدلات الاتية ب١(٨ر٢٨%) ، ب٢ (٨ر٣٠%) ، ج١(٨ر٢٨%) ، ج٢(٦ر١١%). وقد تمت مناقشة هذه النتائج وتأثير الفطريات المعزولة وسمومها وأهميتها بالنسبة للصحة العامة للمستهلك .

SUMMARY

A total of 100 random swab samples were obtained from different meat- cold stores in Ismailia city, 20 from each of the floor, walls, air, roofs and stored meat.All samples were subjected for mycological examination for detection

of mould contamination and aflatoxin production. A total of 482 mould strains could be isolated and identified. The higher frequency percentages were isolated from floor128(26.5%), followed by walls 102 (21.2%), air 89 (18.5%) and roofs 66(13.7%) as well as, from stored meat were 97(20.1%). Twelve mould genera could be detected, where the predominant were Aspergillus 164(34.0%) Cladosporium 91(18.9%) and Penicillium 78 (16.2%). Furthermore nine Aspergillus species were identified, the most of which were A.flavus link 46(28.0%), A.flavus varcolumnaris 41(25.0%), A.niger 28 (17.1%), A.nidulans 18(11.0%) and A.parasiticus 15(3.1%). The isolated Aspergillus flavus group were screened for toxin production . Out of 109 isolates of A.flavus species, 52(47.7%) strains were found to be aflatoxin producers. Aflatoxins B1,B2,G1and G2were secreted by 15(28.8%),16(30.8%),15(28.8%)and 6(11.6%) strains of A-flavus species, respectively.Significance of occurrence and public health importance of the isolated moulds, specially the aflatoxigenic strains were discussed.

Key words: Meat - Aflatoxins - Cold stores

INTROUCTION

Because fungi are ubiquitous in nature,contamination of meat and its products may occur during handling ,storage and subsequent manufacturing(Robb,1993).Such moulds are not only implicated in the spoilage of meat leading to great economic losses but also constitute a major public health hazard due to the production of wide varieties of mycotoxins(Abdel-Rahman and El-Khatib,1989).

Mycotoxins comprise a structurally diverse family of fungal toxins,many of which have been strongly implicated as chemical progenitors of toxicity in man and animal(Ramos et al.1996).

Aflatoxins occupy the most important ingredients among mycotoxins, that is a collective term for a group of structurally similar coumarins, which produced mainly by the common mould A.flavus link and A.parasiticus. There are four major naturally occuring aflatoxins, the most hepatotoxic being aflatoxin B1, and three similar copounds B2,G1, and G2. One of the main aflatoxin B1 biotransformation products as aflatoxin M1(Veldman et al., 1992 and Zaky et al., 1995) .The principal biological effects are carcinogenicity, immunosuppression, mutagenicity and teratogenicity (Betina, 1989). Also aflatoxins may cause liver damage, alteringin lipid

metabolism and they can aid in depression of protein synthesis (Leibetseder , 1981 and Ostrowski , 1984).

Different mould genera were isolated and identified from swab samples obtained from forzen meat and meat-cold stores as water,air, floor and walls.The main isolated mould genera were Aspergillus, Caldosporium, Penicillium,Mucor, Alternaria and Fusarium (Abdel-Rahman et al., 1985, EL-Daly et al., 1988; Mousa et al.,1988; Mansour et al., 1990 and Refai et al., 1993). Among these moulds,Aspergillus species had received a great attention as it predominated in producing aflatoxins which have great puplic health hazards(Lie and Marth,1968 and Hamdy et al.,1993).

Therefore, the present study is carried out to isolate and identify such mould growth with a great attention to the genus Aspergillus, including screening test for determining the aflatoxin-producing strains.

MATERIAL and METHODS

A total of 100 swab specimens were collected from meat cold stores of Ismailia city,20 from each of the floor,walls,air,roofs and stored meat.All samples were mycologically examined as follows :

1- Isolation of mould genera:

The samples were cultured by using malt extract agar and Czapeck Dox-agar media. The isolates were identified according to Raper and Fennel (1965), Zycha et al.(1969), Barnett and Hunter (1972) and Samson (1979).

2- Screening of the aflatoxin-producing aspergilli :

A total of 109 isolates of A.flavus species were inoculated at the centre of solidified fluorescent agar media in glass Petri dishes, then the plates were incubated at 25°C for10 days (according to Hara et al., 1974). The plates were examined under UV lamp for the detection of the fluorescent colour in the agar surrouding colonies.

3-Cutlivation and extraction of aflatoxins :

The toxic strains of A.flavus group that were illuminated in the fluorescent agar,were inoculated in a rice liquid meduim for 15 days at 25 C (Shotwell et al, 1966).

At the end of incubation time extraction of aflatoxins is carried out using chloroform and then concentrated in a rotatory vacuum evaporator.

4- Identification and confirmation of aflatoxins :

The concentrated extracts of A.flavus cultures in rice liquid medium were analysed for identification and confirmation of aflatoxins by application

of thin -layer chromatography (TLC) according to Schuller and Egmond (1981).

RESULTS

The results are recorded in Tables (1,2 and 3)and Figures (1, 2 and 3).

DISCUSSION

Results given in Table (1) reveal that 482 mould strains were isolated from swab samples obtained from meat-cold stores and stored meat in Ismailia city. Higher frequency percentages could be isolated from floors 128(26%) followed by walls 102 (21.2%), air 89(19.5%)and roofs 66(13.6%), as well as from stored meat were 97(20.1%).

The frequency percentages of the isolated mould genera as recorded in Table (1) and Fig(1)show that Aspergillus, Cladosporium and Penicillium were the most predominant 164 (34.0%), 91(18.9%)and 18 (16.2%)respectively.While Rhizopus was 32 (6.6%), Alternaria 22 (4.5%), Mucor 22 (4.5%), Paecilomyces 21(4.4%), Absidial 15(3.1%),Fusarium 14 (2.9%), Bortytis 9(1.5%), Geotrichum 7(1.5%)and Trichoderma 7(1.5%). These findings are in agreement with that of Mousa et al.(1988) and Hamdy et al.(1993),while are lower than those reported by Hadlok (1970) and Abdel-Rahman et al. (1985).

The obtained results presented in Table(2) reveal that 164 Aspergillus species could be identified as A.flavus link 46(28.0%), A.flavus varcolumnaris 41(25.0%), A.niger 28 (17.1%) , A.nidulans 18 (11.0%) and A.parasiticus 15(9.2%). Furthermore A.oryzae, A.sydowii and A.repens were isolated but in lower percentages; 7(4.3%), 5(3.0%) and 4(2.4%), respectively. These values are nearly similar to those recorded by Refai and Loot (1969), Hadlock (1970) and Abdel-Rahman et al.(1985).

Table (3) and Fig.(3) showed the aflatoxigenic A.flavus species which were isolated from meat-cold stores and stored meat.Out of 109 Aspergillus species, 52(47.7%) were aflatoxin-producing strains which could be identified as A.flavus link 24(52.2%), Aflavus varcolumnaris 17(41.5%), A.parasiticus 7(46.6%) and A.oryzae 4(57.1%).These toxic isolates provided aflatoxins B1,B2,G1 and G2 with values of 16(28.8%), 15(18.8%)and 6(11.6%), respectively. These findings are more or less in agreement with that of Taber and Schroeder (1969) and Hamdy et al.,(1993).

From the results obtained in the present study, it is achieved that frozen meat and cold-stored chambers are heavily contaminated with mould species (specially toxigenic Aspergilli) which are the main source of contamination either before or during transportation. Such contamination may be attributed to unsatisfacotry hygienic measures adopted in cleaning and disinfecting of meat-cold stores, before and after storge of meat or its products (Abdal-Rahman et al, 1985 and Mousa et al., 1988).

Presence of the aflatoxin-producing fungi in meat-cold stores increases the probability of hazards arising from mould growth on meat. The direct hazard to human health from aflatoxins is achieved due to mycotoxigenic strains are still able to secrete mycotoxins in stored meat at suitable circumstances (Scott, 1973). Therfore, fungal contamination of meat should be controlled by strict hygienic precautions during production, transportation, processing and storage.

REFERENCES

Abdel-Rahman H., Darwish A. and Hamdy M. (1985): Mould affecti- on of meat cold stores. Assiut Vet. Med. J., 12(24): 154-159.

Abdel-Rahman H. and El-Khatib T. (1989): Mould and yeast hazard in frozen ground beef. Assiut Vet .Med J., 129(14): 124-192

Barnett, H.L. and Hunter, B.B. (1972): Illustrated genera of imperfect fungi 2nd (Ed.), Burgess Put. Co.

Betina V. (1989): Mycotoxins: chemical, biological and environmental aspects In Betna (ed.), elsevier Science Publishers, Amsterdam.

Eldaly E., Abdel. Galil Y. and Saleh E. (1988): Studies on food born fungi on carcase surface and air in Zagazig abattoir. The Bul. of The High Institute of Public Health, 18: 1065-1072.

Hadlok, R. (1970): Untersuchung uber Vorkommen, Herkunft und Bedeutung von Schimmelpizen, Insbesondere der Gattung Aspergi-llus Mich ex Fries, bei Fleisch prudukten. Habil. Giessen.

Hamdy M., mansour N., Awad H. and Biomy M. (1993): A study on isolated moulds, with special reference to Aspergillus sp. from surfa-ce of fresh and cold-stored meat . Vet. Med J., Giza, 14(2): 120.

Hara, S., Fennel D. and Hesseltine C. (1974): Aflatoxin- producing Str- ains of A. flavus detected by fluorescence of agar medium under UV ligh. Applied Microbiology, 27(6): 1118-1143.

Leibetseder J. (1981): Mykotoxikosen Ein Kurze Ein fuhrung uber Tierernahrung, 9: 1-10.

Lie J.L.and Marth E.H.(1968): Aflatoxin formation by A.flavus and A.Parasiticus in a casein substrate at different pH values.J.Dairy Sci.,51.1743-1747.

Mansour, N., Hamdy M., Yassien N. and Refai M. (1990): Dematicae-ous hyphomycetes in slaughtered camels,cattle and surrouding at Cairo abattoir.Assuit Vet. Med. J., 24:145-152.

Mousa M.,Edris A.and Yassien M.(1988): Microbiological monitor-ing of meat -cold stores .Alex. J. Vet. Sci., 4(1):203-213.

Ostrowski-Meissmer H.T. (1984): Effects of contamination of foods by A.flavus on the nutritive value of protein J.Food Sci.Agric.,35(1):47-58.

Ramos, A.J., Gremmels J.F. and Hernandez E. (1996): Prevention of toxic effects of mycotoxins by means of non- nutritive adsorbent compounds .J.Food Protection, 59(6):631-614.

Raper K.B. and Fennel, D. (1965): The genus Aspergillus . 1st (Ed.) , The Williams and Willkans Co.,Baltimore.

Refai M. and Loot, A (1969): Studies of mould contamination of meat in slaughter houses ,butcher`s shops and in cold stores.Mycosen , 12 (10): 621-624 .

Refai M., Mansour N., El-Naggar, A. and Abdel-Aziz, A. (1993): Fungal flora in modern Egyptian abattoirs.Fleischwirtsch.,73(2):172-174.

Robb, J. (1993): Mycotoxins :Contamination and decontamination. J . Feed Mix ,1 (2) 18 - 23 .

Samson, R.A. (1979): Aspergilli described since 1965. Studies in Myc- ology No.18.

Schuller, P. Land Egmond van, H.P. (1981): Detection and determin- ation of mycotoxins in food and feed ,Workshop on mycotoxin ana- lysis, Cairo, 1980, Egypt.

Scott, P.M. (1973): Mycotoxins in stored grains and other cereal prod-ucts.In Grain storage R.N.Sinha and W.E.Muir(Editors).AVI Pub-lishing,Westport, CT.

Shotwell O.L., Hesseltine C.W., Stibblefied R.D. and Sarsenson W. G. (1966): Production of aflatoxin in rice .Appl. Microbiol., 14:425.

Taber, R; A. and Schroeder, H.W (1969): Aflatoxin producing potential isolates of Aspergillus-oryzae group From peanuts. Appl.Microbiol. 15:140.

Veldman A., Meijs J.A., Borggreve G.J. and Heeres-Vander Tol. J.J. (1992): Carry over of aflatoxin from cows food to milk.Animal Prod., 55 :164 - 168 .

Zaky Z., Ismail M. and Refaie R. (1995): Aspergillus flavus and aflatoxin residues in luncheon meat.Assiut Vet.Med .J.,33(66):114-118.

Zycha, H., Siepmann, R. and Linnemann, G. (1969): Mucorales , Eine Beschribung aller Gattungen und Arten dieser Pilzgruppe.,Verlag Von J. Gramer. (Geramany) .

Table (1): Isolated mould genera from meat cold – stores

Mould genera	Floor		Walls		Air		Roof		Meat		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
Aspergillus	48	37.5	37	36.3	28	31.4	19	28.8	32	32.9	164	34.0
Cladosporium	26	20.3	19	18.6	10	11.2	21	31.8	15	15.5	091	18.9
Penicillium	13	10.2	15	14.7	30	33.7	00	00.0	20	20.6	078	16.2
Rhizopus	14	10.9	08	07.9	03	03.4	00	00.0	07	07.2	032	06.6
Alternaria	03	02.3	04	03.9	07	07.9	00	00.0	08	08.3	022	04.5
Mucor	09	07.0	00	00.0	05	05.6	08	12.1	00	00.0	022	04.5
Paecilomyces	06	04.7	05	04.9	00	00.0	04	06.1	06	06.2	021	04.4
Absidia	00	00.0	09	08.8	00	00.0	06	09.1	00	00.0	015	03.1
Fusarium	07	05.0	00	00.0	02	02.3	00	00.0	05	05.2	014	02.9
Botrytis	00	00.0	01	01.0	03	03.4	05	07.6	00	00.0	009	01.9
Geotrichum	00	00.0	04	03.9	00	00.0	03	04.5	00	00.0	007	01.5
Trichoderma	02	01.6	00	00.0	01	01.1	00	00.0	04	04.1	007	01.5
Total & percentage	128	26.5	102	21.2	89	18.5	66	13.7	97	20.1	482	100

Table (2) : Isolated Aspergillus species from meat – cold stores

Aspergillus spp.	Floor		Wall		Air		Roof		Meat		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
A.flavus – link	13	27.1	12	32.4	07	25.0	04	21.0	10	31.3	46	28.0
A.flavus varcolumnaris	11	22.9	08	21.6	09	32.1	06	31.6	07	21.9	41	25.0
A.niger	09	18.7	07	18.9	05	17.9	03	15.8	04	12.5	28	17.1
A.nidulans	04	08.3	03	08.1	02	07.1	05	26.3	04	12.5	18	11.0
A.parasiticus	06	12.5	04	10.9	03	10.7	00	00.0	02	06.3	15	09.2
A.oryzae	03	06.3	00	00.0	01	03.6	00	00.0	03	09.2	07	04.3
A.sydowii	00	00.0	02	05.4	01	03.6	00	00.0	02	06.2	05	03.0
A.repens	02	04.2	01	02.7	00	00.0	01	05.3	00	00.0	04	02.4
Total & percentage	48	29.2	37	22.6	28	17.1	19	11.6	32	19.5	164	100

Table (3) : Isolated Aflatoxin – producing strains of A.flavus group.

A.flavus group	Total	Toxic strains		AFB_1		AFB_2		AFG_1		AFG_2	
	No.	+ ve	%	No.	%	No.	%	No.	%	No.	%
A.flavus link	46	24	52.2	09	37.5	07	29.2	06	25.0	02	08.3
A.flavus varcolumnaris	41	17	41.5	04	23.5	06	35.3	04	32.5	03	17.7
A.parasiticus	15	07	46.6	02	28.6	02	28.6	03	42.8	00	00.0
A.oryzae	07	04	57.1	00	00.0	01	25.0	02	50.0	01	25.0
TOTAL	109	52	47.7	15	28.8	16	30.8	15	28.8	06	11.6

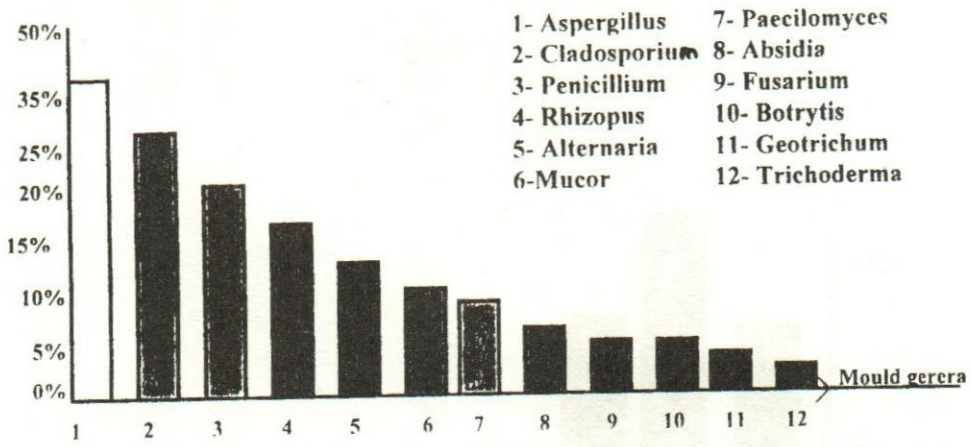


Fig.(1) Frequency of mould genera isolated from meat cold stores and stored meat

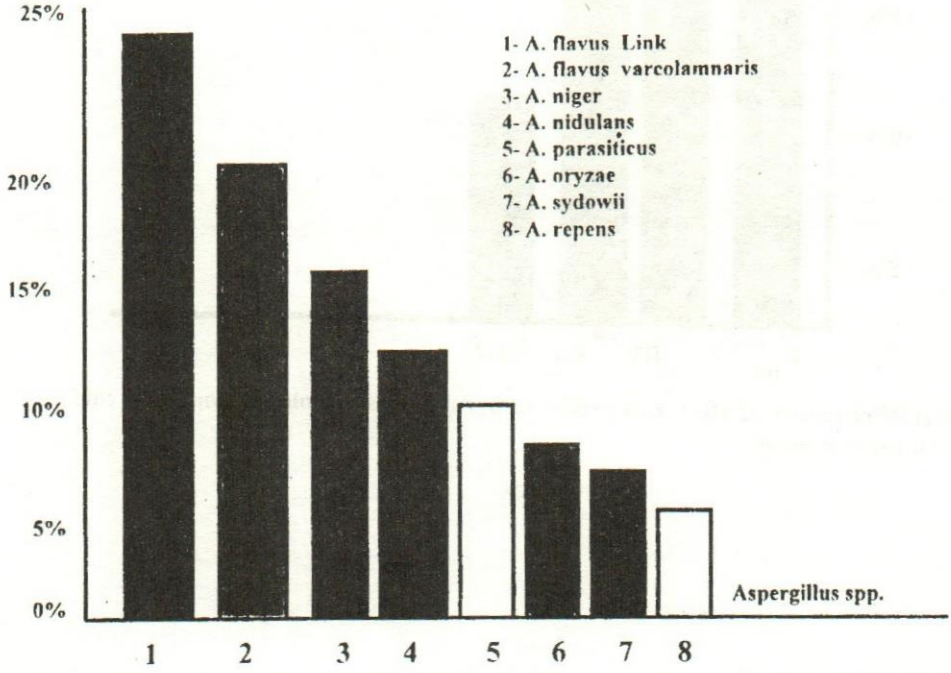
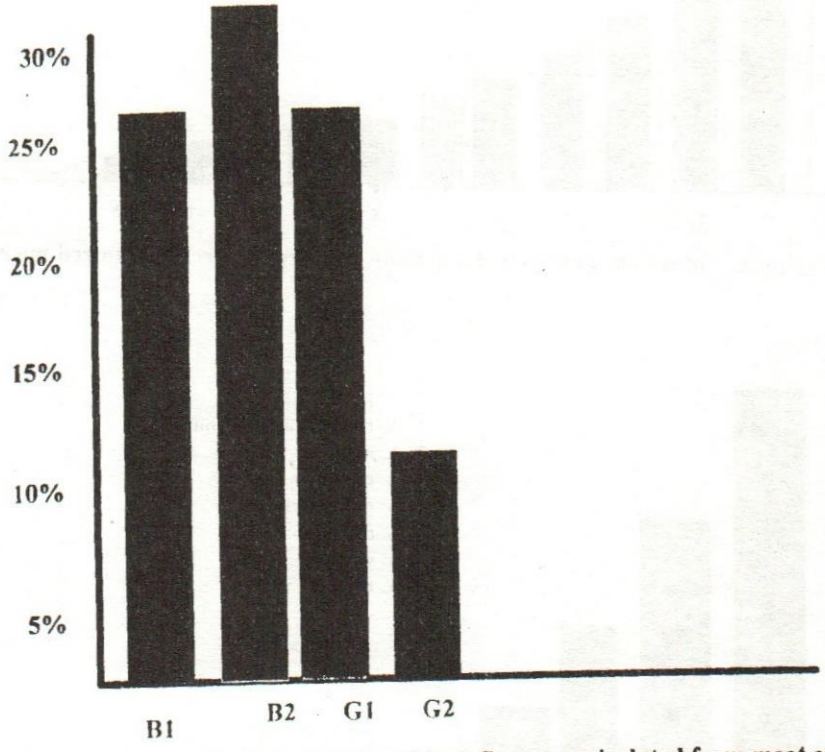


Fig.(2) Frequency of Aspergillus species isolated from meat cold stores and stored meat



Fig(3)Frequency of aflatoxins produced byA.flavus spp.isolated from meat cold stores and meat