

## Evaluation of the mycological status of luncheon meat with special reference to aflatoxigenic moulds and aflatoxin residues

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#### Abstract

The luncheon meat samples analyzed, which were produced locally by the two main luncheon meat producing companies in Egypt were relatively highly contaminated either by moulds and yeasts in general, aflatoxigenic species and aflatoxin residues in particular. The most frequently encountered fungi from the samples were yeasts, *Aspergillus niger, A. flavus, Penicillium chrysogenum, Rhizopus stolonifer, Mucor circinelloides.* Less common were *Cladosporium sphaerospermum, Alternaria alternata, Mycosphaerella tassiana, P. aurantiogriseum* and *P. oxalicum.* The most important aflatoxigenic species, *A. flavus,* was isolated frequently. It was 10% of the total fungal isolates from both samples of the two companies. Seven luncheon meat samples out of 50 analyzed were positive for aflatoxin B<sub>1</sub> or B<sub>1</sub> and G<sub>1</sub>, while all samples were negative for aflatoxins B<sub>2</sub>, G<sub>2</sub>, M<sub>1</sub> and M<sub>2</sub>. Aflatoxin B<sub>1</sub> was detected only in 4 and 3 samples out of 25 analyzed from each of company A and B, respectively. The highest detectable level, 11.1 ppb, was recorded in a sample from company B and the least, 0.5 ppb, in a sample from company A. Aflatoxin G<sub>1</sub>, at concentration of 3.2 ppb, was detected in only one sample of the aflatoxin B<sub>1</sub> – contaminated 3 samples of company B: this sample also had the highest level of aflatoxin B<sub>1</sub>. Some luncheon meat samples had higher numbers of aflatoxigenic *A. flavus* than others, however these samples were negative for aflatoxin B<sub>1</sub>. Some luncheon meat samples had higher numbers of aflatoxigenic *A. flavus* than others, however these samples were negative for aflatoxin.

Key words: aflatoxin residues, Aspergillus flavus, luncheon meat, moulds

#### Introduction

Moulds can contaminate and cause considerable economic loss through spoilage and discoloration of foods. Also, world attention had been focused in the last three decades on the toxicity of some moulds, which are a considerable hazard to health associated with liver damage and carcinogenicity [1].

Aflatoxigenic moulds were first reported in 1963 to be present in U.S. agricultural commodities used for human food [2].

Toxic mould metabolites, mycotoxins, are a broad spectrum of biologically active substances that occur as a result of growth of saprophytic moulds on various types of feed and foods. Aflatoxins are the most important mycotoxins and pose a quadruple threat to both human and animals as they produce four distinct effects: acute liver damage, liver cirrhosis, induction of tumors and teratogenic effects [3–6]. Residues of these toxins in animal tissues and their products is a public health concern.

Luncheon meats usually consist of finely chopped meat and fat with or without some added cereals, cured with salt and nitrite and heat processed [7].

In Egypt, information concerning moulds, aflatoxigenic moulds and aflatoxins from animal products is relatively incomplete. Therefore, this investigation was planned to determine the contamination level of luncheon meat, a product widely consumed in Egypt, by spoilage fungi and yeasts with special reference to aflatoxigenic species and aflatoxin residues.

### Materials and methods

Fifty random samples of luncheon meat were collected from Assiut city markets. These were locally produced by the two main luncheon meat-producing companies in Egypt, coded herein by A and B, with 25 samples from each. The samples were kept in the freezer in separate clean polyethylene bags until mycological and aflatoxin analyses were conducted.

#### Mycological analysis

For detecting, enumerating and isolating fungi, all luncheon samples have been examined on dichloranrose bengal medium of King et al. [8] using direct plating technique [6] as follows: 15 segments, approximately 10 mm square each, from each luncheon meat sample were placed asceptically on the surface of the agar medium, 5 segments per plate. The plates were then incubated at 25 °C for 7–10 days. The fungal colonies were counted, isolated and identified. The identification were based on macroscopic and microscopic characteristics according to [6, 9–18].

#### Aflatoxin analysis

Fifty grams of each luncheon meat sample was minced asceptically and separately and analyzed for aflatoxin residues by the method of the Association of Official Analytical Chemists [19]. Statistical analysis of the data was performed according to Kalton [20].

#### **Results and discussions**

# Spoilage mycoflora associated with luncheon meat samples

Twenty seven genera and 46 species were isolated from 50 luncheon meat samples from the two major producing-companies in Egypt; 22 genera and 36 species of moulds from samples of company A and 20 genera and 33 species from samples of company B (Table 1).

The mean, standard deviation, minimum and maximum numbers of isolates of the most commonly encountered genera and species from luncheon meat of the two companies under investigation are presented in Table 2. At the same time, the percentages for the number of contaminated luncheon meat segments are also presented in Table 2. The number of isolates of fungi and the most common species in the individual samples are shown in Table 3.

Aspergillus species, 21.5% and 26.3% of the total isolates recovered from the samples of the two companies A and B, respectively, *Penicillium* species, 18.7 and 12.6, and yeasts, 23.3 and 25.1, were isolated in high frequencies. *Aspergillus* species were recorded in 100% and 100% of the samples from the two companies, *Pencillium* species 96% and 88%; and yeasts from 84% and 88% of the samples of the two companies, respectively. Similar results have been reported by Abdel-Rahman et al. [21] who found that *Penicillium*, yeasts and *Aspergillus* were the most encountered from luncheon meat.

A. flavus, 10.3% and 10.5% of the total isolates, and A. niger (9.8% and 13.2%) were the most commonly encountered species of Aspergillus. Of Penicillium species only P. chrysogenum, 12.2% and 7.1%, was isolated at a high frequency; however, P. aurantiogriseum, 3.4% and 1.6%, was found moderately in samples of both companies. P. oxalicum isolates were of low occurrence in samples of company A and of moderate occurrence in samples from company B. The remaining 6 Aspergillus species and 5 Penicillium species were less frequent (Table 1). Some of these species were only isolated from luncheon meat of company A, A. oryzae, P. janczewskii, P. solitum, P. variabile, while other species, A. melleus and A. sydowii, were frequent only from luncheon meat of company B.

Abdel-Rahman et al. [21] isolated only 3 Aspergillus species of which A. flavus was the most common followed by A. niger and A. sydowii. However, they isolated 7 Penicillium species of which P. verrucosum var. cyclopium (= P. aurantiogriseum), P. citrinum, and P. italicum were the most common. Roushdy et al. [22] obtained a few fungi from luncheon meat, Aspergillus niger and A. flavus being the most common, also Penicillium verrucosum, Mucor spp. and A. ochraceus. Many Aspergillus and Penicillium species have been previously reported from other meat products such as minced meat and basterma [21], sausages [23–27], and frozen meat as well [28–30]. Yeasts also a considerable component of the mycoflora of minced meat and basterma [21] as well as fermented sausages [27, 31].

Some other fungi were isolated in high frequency from samples of company B, Alternaria (A. alternata), Cladosporium (C. sphaerospermum) and Rhizopus (R. stolonifer). Another species of Alternaria, A. tenuissima, was rarely encountered from company A

Table 1. Fungi associated with 50 luncheon meat samples from the two producing companies in Egypt\*

Taxa	Comp	oany A			Comp	oany B		
	NI	%I	%F	0	NI	%I	%F	C
Acremonium strictum W. Gams	2	0.32	8	R	4	0.59	8	R
Actinomucor elegans (Eidam) C.R. Ben & Hesseltine	8	1.29	4	R				
Alternaria	31	4.98	60	Н	31	4.60	44	Ν
A. alternata (Fries) Keissler	27	4.34	52	Н	31	4.60	44	Ν
A. tenuissima (Kunze) Wiltshire	4	0.64	8	R				
Aspergillus	134	21.54	100	Н	177	26.26	100	ł
A. alutaceus Berk. & Curtis	2	0.32	8	R	1	0.15	4	I
A. <i>flavus</i> Link	64	10.29	84	Н	71	10.53	84	I
A. fumigatus Fres.	4	0.64	12	R	8	1.19	20	Ι
A. melleus Yukawa					1	0.15	4	I
A. niger van Tieghem	61	9.81	88	Н	89	13.21	92	ł
A. oryzae (Ahlb.) Cohn	2	0.32	4	R				
A. sydowii (Bain. & Sart.) Thom & Church					6	0.89	24	Ι
A. <i>terreus</i> Thom	1	0.16	4	R	1	0.15	4	I
Candida parapsilosis (Ashford) Langeron & Talice	11	1.77	4	R				
Cladosporium	27	4.34	52	Н	43	6.38	48	I
C. cladosporioides (Fres.) de Vries					5	0.74	4	J
C. sphaerospermum Penz.	27	4.34	52	Н	38	5.64	44	l
Cochliobolus	6	0.97	24	L	1	0.15	4	]
C. lunatus Nelson & Haasis	1	0.16	4	R	1	0.15	4	]
C spicifer Nelson	5	0.80	20	L				
Colletotrichum dematium (Pers.) Grove					1	0.15	4	]
Curvularia ovoidea (Hiroe & Watan.) Muntanola	2	0.32	4	R				
Emericella	1	0.16	4	R	4	0.59	12	J
E. nidulans (Eidam) Vuillemin	1	0.16	4	R	3	0.45	12	J
E. quadrilineata (Thom & Raper) C.R. Benjamin					1	0.15	4	]
Epicoccum nigrum Link	2	0.32	8	R	5	0.74	20	I
Fennellia flavipes Willey & Simmons	2	0.32	8	R				
Geotrichum candidum Link					2	0.30	4	I
Gibberella fujikuroi (Sawada) Ito					2	0.30	8	I
Mucor	45	7.24	40	М	53	7.86	40	I
M. circinelloides van Tieghem	45	7.24	40	М	46	6.83	24	I
<i>M. racemosus</i> Fres.					7	1.04	24	I
Mycosphaerella tassiana (De Not) Johanson	16	2.57	24	L	27	4.01	28	ľ
Nectria haematococca Berk. & Brown	1	0.16	4	R	1	0.15	4	I
Paecilomyces variotii Bainier	2	0.32	4	R				
Penicillium	116	18.65	96	Н	85	12.61	88	I
P. aurantiogriseum Dierckx	21	3.38	48	М	11	1.63	28	l
P. chrysogenum Thom	76	12.22	84	Н	48	7.12	76	1
P. hirsutum Dierckx	3	0.48	12	R	1	0.15	4	I
P. janczewskii Zaleski	1	0.16	4	R				
P. oxalicum Currie & Thom	11	1.77	24	L	10	1.48	32	l
P. solitum Westl.	1	0.16	4	R				
P. variabile Sopp	1	0.16	4	R				
P. viridicatum Westl.	2	0.32	8	R	15	2.23	4	I
Pleospora tarda E. Simmons			-		10	1.48	16	1
Rhizopus stolonifer (Ehren.) Lind	57	9.1	56	Н	34	5.05	28	1
Rhodotorulua mucilaginosa (A. Jorg.) F.C. Harrison	4	0.64	4	R	4	0.59	4	I
Syncephalastrum racemosum (Cohn) Schroeter	6	0.97	8	R	4	0.59	4	I

Таха	Com	oany A			Com	oany B		
	NI	%I	%F	0	NI	%I	%F	0
Trichoderma harzianum Rifai					5	0.74	8	R
Trichosporon cutaneum (de Beurm., Goug.& Vauch.) Ota	2	0.32	8	R	12	1.78	24	L
Ulocladium chartarum (Preuss) E. Simmons	1	0.16	4	R				
Yeasts	145	23.31	84	Н	169	25.08	88	Н
Zygorrhynchus heterogamous (Vuill.) Vuill.	1	0.16	4	R				
Total isolates	622	100	100		674	100	100	
Total number of genera (27)	22				20			
Total number of species (46)	36				33			

\*NI = Number of isolates (calculated per 375 segments).

%I = Percentage isolates (calculated per total fungal isolates of 25 samples).

%F = Percentage frequency (calculated per total luncheon samples, 25 from each company).

O = Occurrence: H = high 50-100% of the cases, M = moderate 25-49% of the cases, L = low, 13-24\% of the cases, R = rare, 1-12% of the cases.

samples only. Other *Cladosporium* species (*C. cladosporioides*) was also rarely encountered but only from company B samples. Out of the three genera mentioned above, only *Cladosporium* was obtained and less frequent from luncheon meat [21] and from other meat products such as sausage, beefburger, basterma, minced meat and frozen meat [21, 22]. However, *Alternaria* (*A. alternata*) has been recorded from cold stored and frozen meat [30, 32, 33]. *R. stolonifer* also was reported previously from meat products [30, 34].

Two other genera were encountered in moderate occurrence from samples of company B; *Mucor* with *M. circinelloides* being the most frequent species and *Mycosphaerella* with *M. tassiana* the most common species. *Mucor* species were moderately isolated and *Mycosphaerella* species in low frequency from samples of company A. Only *Mucor* was isolated less frequently from luncheon meat [21, 22], sausage, beefburger, minced meat, frozen meat [22, 23], as well as from cold stored meat [28, 33]. *Mucor circinelloides* has been reported from meat [34]. Also, *M. racemosus* has been found contaminating frozen and processed meats [30, 35] and beef carcasses [36].

The remainder of the fungus species were infrequently isolated either from samples of company A (Actinomucor elegans, Candida parapsilosis, Cochliobolus spicifer, Curvularia ovoidea, Fennellia flavipes, Paecilomyces variotii, Ulocladium chartarum, Zygorrhynchus heterogamous); or company B (Colletotrichum dematium, Emericella quadrilineata, Geotrichum candidum, Gibberella fujikuroi, Pleospora tarda, Trichoderma harzianum) or from samples of both luncheon meat-producing companies (Acremonium strictum, Cochilobolus lunatus, Emericella nidulans, Epicoccum nigrum, Nectria haematococca, Rhodotorula mucilaginosa, Syncephalastrum racemosum and Trichosporon cutaneum). Of these fungi, Geotrichum candidum and Trichosporon had been reported from luncheon meat; however Rhodotorula was isolated from other meat products (minced meat and basterma) [21]. Most of these fungi were isolated from other meat products, sausage [23], frozen meat [28–30] and from beef carcases as well [33, 36–39].

#### Aflatoxin residues in luncheon meat samples

Aflatoxin B<sub>2</sub>, G<sub>2</sub>, M<sub>1</sub> and M<sub>2</sub> were not detected in any of the 50 luncheon meat samples analyzed. However, only 7 luncheon meat samples out of 50 tested, 4 from company A and 3 from company B, were found to be contaminated by aflatoxin B<sub>1</sub>, and only one of the three aflatoxin  $B_1$  – contaminated samples from company B was also contaminated with aflatoxin G<sub>1</sub> (Table 3). The highest detectable levels were 11.1 ppb and 3.2 ppb for aflatoxins B1 and G1, respectively. Similar results were obtained by Roushdy et al. [22] who found that only 10%, 2 samples out of 20 analyzed, of luncheon meat samples were contaminated by aflatoxin with a mean concentration of 0.41 ppm. On the other hand, aflatoxins have been reported from fresh meat, frozen meat or other meat products (minced, fresh sausage, beefburger) [22].

Aflatoxins are produced in nature only by *Aspergillus flavus*, *A parasiticus* and *A nomius* [6, 40, 41]. In this study, luncheon meat samples were found

*Table 2.* Count  $[M \pm SD(Mn-Mx)]$  and percentage of the most commonly encountered fungi from the contaminated segment of luncheon meat (%S) of the two companies<sup>\*</sup>

	Company A		Company B	
	$[M \pm SD(M_n - M_x)]$	%S	$[M \pm SD(M_n - M_x)]$	%S
Total	$[24.88 \pm 6.75 (11 - 47)]$		$[26.96 \pm 7.98  (1851)]$	
Yeasts	$[5.8 \pm 4.28 \ (0-13)]$	38.7	$[6.76 \pm 3.82 \ (0-15)]$	45.1
Aspergillus	$[5.36\pm 6.26\ (0\text{-}30)]$	35.7	$[7.08 \pm 5.74 \ (0-27)]$	47.2
A. niger	$[2.44 \pm 3.07 \ (0-14)]$	16.3	[3.56 ± 2.97 (0-12)]	23.7
A. flavus	$[2.56 \pm 3.11 \ (0-14)]$	17.1	$[2.84 \pm 3.35 \ (0-15)]$	18.9
Penicillium	$[4.64 \pm 3.84 \ (0-16)]$	30.9	$[3.4 \pm 3.65 \ (0-17)]$	22.7
P. chrysogenum	$[3.04 \pm 3.13 \ (0-15)]$	20.3	$[1.92 \pm 1.66 \ (0-5)]$	12.8
P. aurantiogriseum	$[0.84 \pm 1.07 \ (0-3)]$	5.6	$[0.44 \pm 0.92 \ (0-4)]$	2.9
P. oxalicum	$[0.44 \pm 0.96 \ (0-4)]$	2.9	$[0.4 \pm 0.71 \ (0-3)]$	2.7
Rhizopus stolonifer	$[2.28 \pm 2.56 \ (0-9)]$	15.2	$[1.36 \pm 2.50 \ (0-8)]$	9.1
Mucor	$[1.8 \pm 3.54 \ (0-14)]$	12	$[2.12 \pm 3.75 \ (0-13)]$	14.1
M. circinelloides	$[1.8 \pm 3.54 \ (0-14)]$	12	$[1.84 \pm 3.85 \ (0-13)]$	12.3
Cladosporium	$[1.08 \pm 1.26 \ (0-4)]$	7.2	$[1.72 \pm 2.37 \ (0-8)]$	11.5
C. sphaerospermum	$[1.08 \pm 1.26 \ (0-4)]$	7.2	$[1.52 \pm 2.29 \ (0-8)]$	10.1
Alternaria	$[1.24 \pm 1.54 \ (05)]$	8.3	$[1.24 \pm 1.94 \ (0-6)]$	8.3
A. alternata	$[1.08 \pm 1.47 \ (05)]$	7.2	$[1.24 \pm 1.94 \ (0-6)]$	8.3
Mycosphaerella tassiana	$[0.64 \pm 1.29 \ (0{-}5)]$	4.3	[1.08 ± 2.34 (0–9)]	7.2

\*[M  $\pm$  SD (M<sub>n</sub>-M<sub>x</sub>)] = M, mean total isolates out of 25 samples.

SD, Standard deviation.

M<sub>n</sub>, minimum number of isolates arising from 15 segments of one of the 25 samples analyzed.

M<sub>x</sub>, maximum number of isolates arising from 15 segments of one of the 25 samples analyzed.

%S: Calculated per total number of segments analyzed (375 from each company).

to be highly contaminated only by *A. flavus* of the aflatoxigenic species. It was isolated from 21 out of 25 luncheon samples examined from each company and accounted for approximately 10% of the total fungal isolates. Some luncheon meat samples contained higher numbers of *A. flavus* isolates than others and these samples were found to be aflatoxin free. These results agree with those obtained by Bullerman [42] and Zohri et al. [43] who reported that the presence of toxigenic moulds in a food product did not automatically mean the presence of mycotoxins, though it indicated the potential for mycotoxin contamination.

Since aflatoxin  $B_1$  is heat stable [44], it is not affected by heating during luncheon meat processing. The possibility exists that contamination of luncheon meat by aflatoxin could have originated either from the animal tissues previously fed on aflatoxin-contaminated feed or by use of aflatoxin – contaminated ingredient, e.g. cereals. Early studies by Allcroft and Carnagham [45] and Keyl et al. [46] on aflatoxin residues in meat tissues of animals fed contaminated feed suggested that no significant buildup of aflatoxins in animal tissues occurred. However, other workers re-

ported that significant quantities of the aflatoxins have been detected in beef cattle [47] fed on aflatoxin contaminated feeds. Feng and Tang [48] found that maize, maize bran, groundnut cakes, and any mixed feed containing maize and groundnut cakes were seriously contaminated with aflatoxins.

In this study, luncheon meat samples from the two main luncheon-producing companies were demonstrated to be contaminated by a wide variety of spoilage fungi and yeasts. Of these fungi, some are known to be mycotoxin producers; A. alutaceus, A. flavus, A. sydowii, Emericella nidulans, Gibberella fujikuroi, P. aurantiogriseum, P. viridicatum. Luncheon meat samples were highly contaminated by aflatoxigenic species A. flavus, and by aflatoxins as well. Such contamination in meat products is a matter of considerable concern as ingestion of low levels of mycotoxins over extended periods constitutes a public health hazard. Contamination probably originated from other additives than animal tissues or during processing, transport and/or storage. Therefore, food additives should be analyzed for mycoflora and mycotoxins especially aflatoxins, before these additives are used in food manufacturing. Also, strict hygienic measures

	Total			а	Aspergillus	gillus	Aspergillus		Clado	Cladosporium	Mucor	2	Penic	Penicillium	Penic	Penicillium			Yeasts		Aflatox	Aflatoxins, ppb
ple	isolat	isolates (TI)	alternata		flavus		niger		sphaer	sphaerospermum	circine	elloides	aura	circinelloides aurantiogriseum	chrys	chrysogenum	stolonifer	ujfer				
No.	A	В	А	B /	A ]	В	A	В	A J	В	Α	В	A	В	А	В	А	В	A	В	А	В
1	25	30	3	1	2	9	2	3	3	4						2	5			9	1.3B1	
																						3.2G1
5	47	25	S	Ś	14	ε	14	6					ε		S	1	ε	4		$\mathfrak{c}$		
33	27	32		9	ю		1	ю		2			-		15	1	1	5	9	10		
4	34	27	1	7	9	6	ю	8	ю				-		6		5		8	×		
5	32	51		5	8	15	9	12						1	З	5	1			5		
9	24	27	1		1		1	7	7	4						1			×	6		
7	11	21		1		1	1	7	1	1					1	ю		8	٢			
8	20	27	1		1	7	0	7	1	9									10	٢		
6	22	33	1	ŝ	0	0	1	0							1	7		4	13	×		
10	25	27		1	1		0	ŝ	7	4	1				0	1			6	13		
11	25	30	7		1	ю	5		4	1					1		ю	5	9	15		2.5B1
12	20	23		1	1	1	-	8	б			4			-			٢	13	-	1.6B1	
13	21	32	1	1	б		2			5					З	7			12	5		
14	25	46	1	2		9	1	10	7	8	10			4	З	ŝ	4	1	0	9		
15	17	22				1		1		2	14	13	1		1	0			1			
16	24	28	1		0	1	1	4		1	1	9	0	2	4	б	6		0	6		
17	35	23	4		4	б	1	ŝ	7		0	13			5	4	5		11			
18	24	18	4		4	1	8	1					0	1	0	-				10		
19	22	19			5	4	ю	4					-				5		9	×		
20	25	20			1	1	1	4	1				-		0	ю	0		6	9	2.3B1	2.0B1
21	25	28	0		1	ю		ю			4	5	ю	1	4	5	5		4	×	0.5B1	
5	23	18			1	1		1	1		ю		7	1	9	1			٢	10		
3	25	19			1	4	1	4	0		0		ю		S		5		0	×		
4	20	20				1	ю	4			1		-		5	б	4		7	9		
25	24	28			0	ю	1	ю			٢	5		1	5	S			٢	×		
Ι	622	674	27	31 (	2	71	61	68	27	38		46	21	11	76	48	57	34	145	169		
1%	100	100	4.3	4.6	10.3	10.5	9.8	13.2	4.3	5.6	7.2	6.8	3.4	1.6	12.2	7.1	9.2	5.1	23.3	25.1		
%Е	100	100	5	5	10	5	00		0		0		0	00		ì	ì	00		00		

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