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Research Paper

MYCOLOGICAL PROFILE OF FROZEN MEAT WITH SPECIAL REFERENCE TO AFLATOXIN B_1 AND OCHRATOXIN A RESIDUES IN FROZEN MEAT

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Abstract

A total of 100 samples of meat cuts and minced meat (50 of each) were collected from different localities of different sanitation levels from Gharbia governorate. For moulds, the prevalence of isolated species were Aspergillus (52.9% and 45.6%), Penicillium (21.1% and 27.2%), Acremonium (3.5 % and 6.8%), Alternaria (11.7% and 12.6%), and Curvularia (4.7% and 0.9%) for meat cuts and minced meat respectively. Endomyces and Eupenicillium were isolated by a percent of (2.3% and 3.5%) respectively for meat cuts, while Chaetomium and fusarium were (3.9% and 2.9%) for minced meat respectively. The result showed that the Aspergillus and Penicillium were the most isolated mould species, furthermore Aspergillus was further identified to *A.flavus, A.niger, A.terreus, A. aculeatus* and *A.fumigatus*. *A. niger* was the most predominant specie (48.8% and 59.5%) for meat cuts and minced meat respectively. Penicillium also was further identified to *P. fellutanum, P .citreonigrum, P. aurantiogriseum* and *P. thomii. P. aurantiogriseum* was the most predominant species (33.3% and 32.1%) for meat cuts and minced meat respectively. For yeasts, the prevalence of isolated species were Candida (50% and 48.4%), Rhodotorula (13.3% and 18.7%), Saccharomyces (16.6% and 12.5%) and Torulopsis (20% and 15.6%) for meat cuts and minced meat respectively. The results also indicated the mean concentration of aflatoxin B1 was (7.8±2.2 and 3.4±0.5) and Ochratoxin A (3.9±0.8 and 8.1±1.9) for meat cuts and minced meat respectively.

Key words: mould, yeast, aflatoxin B_1, ochratoxin A, frozen meat.

INTRODUCTION

The increased population in our country Egypt requires a parallel raise in the field of food production. Especially meat and its products so the demand of the meat products increased with a mass production level in the last few years, so imported frozen meat is a necessity in order to solve national meat demand in Egypt. It is used mainly as a raw material in manufacturing meat products.

Moulds, tend to grow on the surface of objects in the shape of a visible 'mycelium' made up of many cells. Moulds have both positive and negative effects on the food industry. Specific types of moulds are beneficial for the food industry since these are involved in Blue, Brie, Camembert, and Gorgonzola cheeses. Other types of moulds can be quite toxic and may produce allergic reactions and respiratory problems, or produce poisonous substances called mycotoxins. Aspergillus mould, for instance, which is most often found on meat (as well as in environment), can cause an infection called Aspergillosis, which is actually a group of illnesses ranging from mild to severe lung infections, or even whole-body infections. One of the greatest concerns regarding mould in food is the mycotoxins that some varieties produce. One of the most researched mycotoxins is aflatoxin, a cancer-causing poison **[1].**

Moulds only compete with bacteria on meat when storage temperatures are lowered to 8^0C or below, or when theme at surface dries to an a_w that enables fungi to compete. In earlier literature, spoilage of chilled or frozen meat by fungi was usually attributed to Mucorales, especially *Thamnidium elegan*s and Mucor species which grew as ''whiskers'' on cold stored meat. The most commonly isolated fungi from beef aging at refrigeration temperature were *Thamnidium elegans, Mucor mucedo* and *Helicostylum pulchrum* and *Chaetostylum fresenii Tiegh*. Also, reported that very infrequent isolation of Mucorales from meat and questioned their significance, but it seems more probable that techniques for meat storage have changed rather than that the prewar meat technologists were wrong **[2].**

Mould and yeast contamination in imported frozen meat may be attributed to poor sanitary measures during production, transportation, storage and handling of frozen meat from time of their arrival sea port until it reached different retail cold stores **[3].**

Mycotoxin is the general name of the toxic compounds produced by moulds. Among the other chemical agents found in food and feedstuffs, mycotoxins are the very important ones that threaten human and animal health. In this point of view; mycotoxin contamination of meat is one of the important hazards to be controlled in terms of food safety **[4]**. However it is impossible to fully calculate, it is suggested that mycotoxin contamination is the reason of big economic losses as well as food safety and public health problems **[5].**

Mycotoxins are transported to humans by consumption of meat obtained from animals fed with contaminated feeds and contaminated spices used during processing of these meats. Mycotoxins are thought to be responsible for various pathological syndromes in humans. For example, ochratoxin is associated with Balkan endemic nephropathy and fumonisin B_1 is associated with esophagus cancer International Agency for Research on Cancer **[6].**

Therefore, this investigation was planned for is to investigate the mycological status aflatoxin B_1 and ochratoxin A of frozen meat sold in popular markets.

2. MATERIAL AND METHODS

2.1.Collection of Samples:

A total of 100 frozen meat samples (meat cut and minced meat) 50 of each were obtained randomly from main markets and shops at Gharbia Governorate and labelled appropriately. The collected samples were placed in sterile ice-packed containers and conveyed to the laboratory without delaying for analysis.

-Preparation of samples: The collected samples were prepared according to the technique recommended by **[7]** and Determination of total mould and yeast counts according to **[8].**

2.2. Identification of mould isolates according to **[9].**

2.3. Identification of yeast isolates according to **[10]** and by using Rapid ID yeast plus system **[11].**

2.4. Detection of aflatoxin B_1 residues in frozen meat samples by using fluorimeter method: according to **[12]**.

2.5. Detection of ochratoxin A residues in frozen meat samples by using fluorimeter method: according to **[13].**

3. RESULTS AND DISCUSSION

Moulds only compete with bacteria on meat when storage temperatures are lowered to 8^0C or below, or when theme at surface dries to an a_w that enables fungi to compete. In earlier literature, spoilage of chilled or frozen meat by fungi was usually attributed to Mucorales, especially T*hamnidium elegans* and Mucor species which grew as ‘‘whiskers’’ on cold stored meat. The most commonly isolated fungi from beef aging at refrigeration temperature were *Thamnidium elegans, Mucor mucedo* and *Helicostylum pulchrum* and *Chaetostylum fresenii Tiegh.* Also, reported that very infrequent isolation of Mucorales from meat and questioned their significance, but it seems more probable that techniques for meat storage have changed rather than that the prewar meat technologists were wrong **[2].**

Mould contamination of meat and meat products may occur during slaughtering of the animals, transportation, or during processing of meat products through the use of contaminated equipments or contaminated additives and spices which considered the most important source of mould contamination in meat products **[14]**.Result obtained in Table (1) indicated that the incidence of mould and yeast in the examined meat samples were 86%and 100% for frozen meat cuts and minced meat. Also, from our results revealed that the total mould and yeast count ranged from 1x10 to $5.4x10^4$ and 1x10 to $4.1x10^5$ (cfu/g) with the mean value of $4.5x10^3 \pm 1.3x10^3$ and $2.8x10^4 \pm 9.6x10^3$(cfu/g) for meat cuts and minced meat respectively. From the above mentioned results, it is obvious that the most of samples of the mould and yeast counts were higher than that recommended by **[15]**. The results in table (1) revealed that the nearly similar results were obtained by **[3]** who recorded that the incidence of mould and yeast in frozen meat were (92%) and (94%) respectively, while **[16]** recorded that the incidence of mould and yeast were (57%) and (43%) respectively. For minced meat our results are nearly similar to that mentioned by [17] that the incidence of mould in minced meat was (100%), while lower incidence was recorded by **[18]** who

illustrated the frequency distribution of mould and yeast were (47.6%) and (53.8%) respectively. The difference between results may be attributed to the contamination level in the abattoir environment and the hygienic measures adopted during butchering, transportation, storage and meat mincing.

The results in table (1) revealed that the total mould and yeast count of examined frozen meat cuts Similar results obtained by **[19, 20 ,21 and 22],** higher results for frozen meat were obtained by **[23]** who stated that the mean count of mould and yeast in frozen meat were $1.22x10^4 \pm 2.07x10^3$cfu/g. and $2.85x10^3 \pm 3.99x10^2$ respectively. The difference of results attributed to the temperature and humidity during storage and the hygienic measures adopted in cold stores and markets. On the other hand similar results was obtained by **[17]** who recorded that the mean count of total mould and yeast for minced meat was $1.6x10^4$. Mostly the reason of minced meat contamination might be due to the poor hygienic quality of raw meat, inadequate storage and thawing conditions, contamination form grinder and the time between mincing and using **[24]**. From the above mentioned results, according to the safe permissible limits stipulated by **[15]** for total mould and yeast (fungi) count in meat products (Free), in case of frozen meat cuts it was indicated that, 86% the examined samples of meat products were not accordance with these limits .While, in examined frozen minced meat not mentioned anything about fungi according to the permissible limits stipulated by **[25].**

According to the safe permissible limits stipulated by [14] for total mould and yeast (fungi) count in case of frozen meat cuts it was indicated that, 86% the examined samples of meat products were not accordance with these limits .While, in examined frozen minced meat not mentioned anything about fungi according to the permissible limits stipulated by **[15].**

Results showed in table (2) showed that there were a significant variation of the total mould and yeast count between samples, this variation may be due to the contamination occurred during the processing of minced meat.

The result tabulated in table (3) illustrated that Aspergillus was further identified to *A.flavus* (40%&21.2%), *A.niger,* (48.8% and 59.5%) *A. terreus* (11.1%&6.4%), *A. aculeatus* (0%&4.3%) *and A.fumigatus* (0%& 8.5%) for meat cuts and

minced meat respectively. *A. niger* was the most predominant species (48.8% and 59.5%) for meat cuts and minced meat respectively. Penicillium also was further identified to *P. fellutanum* (27.7% and 14.3%), *P. citreonigrum* (11.1% and 25%), and *P. thomii* (27.7% and 28.5%).*P. aurantiogriseum* was the most predominant species (33.3% and 32.1%) for meat cuts and minced meat respectively.

From the results given in table (3) revealed that frozen cut meat and minced meat samples were contaminated with many fungal genera and species. The mould species isolated from frozen cut meat and minced meat *Acremonium strictum, Alternaria alternate, Curvularia lunata* (100%&100), While *Endomyces fibuliger and Eupenicillium. Javanicum* isolated only from frozen cut meat. *Chaetomonium brasiliense* and *Fusarium verticillioides* were isolated only from minced meat samples.

The results obtained in table (4) indicated that the prevalence of isolated yeast species were Candida (50% and 48.4%), Rhodotorula (13.3% and 18.7%), Saccharomyces (16.6% and 12.5%) and Torulopsis (20% and 15.6%) for meat cuts and minced meat respectively. Ustilaga (4.7%) isolated only from minced meat. The result showed that the Candida was the most isolated yeast species.

Table (5) showed that Candida was further identified to 6 species *C. guilliermondii* was the predominant specie (46.6% and 35.4%), *C. albicans* (6.6% and 6.5%), *C. tropicalis* (13.3%and 9.7%), *C. krusei* (13.3%and 9.7%) and *C.parapsilosis* (20%and 22.6%) for meat cuts and minced meat respectively. *C. valida* (16.1%) in minced meat. Also, Rhodotorula was identified to 2 species *R. mucilaginosa* (100%) and 83.3%) for meat cuts and minced meat respectively. *R. glutinis* (16.7%) in minced meat. The data obtained in tables (3), (4) and (5) of mould identification declared that the most predominant mould genera in examined meat samples were; Aspergillus, Penicillium, Acremonium spp., Alternaria spp., Chaetomium spp., Curvularia spp., Endomyces spp., Eupenicillium spp., Fusarium spp. , candida spp., Rhodotorula spp., Saccharomyces spp. and Torulopsis spp. which agree with the results obtained by many researchers as **[26, 27, 28 , 29 and 30]** who recorded that the most isolated fungal genera from examined minced meat were Aspergillus, Penicillium, Cladosporium, Mucor, Acremonium and Fusarium. Nearly results mentioned by **[31]** and **[29]** who recorded that *A. niger* had higher frequency occurrence among Aspergillus species from

the examine meat samples. While in case yeast species. Nearly similar results recorded by **[32, 3 and 29]** who isolated Candida spp., Rhodotorula ssp., and saccharomyces while **[17]** isolated Torulopsis ssp. from minced meat. Most meat spoilage by mould strains survived freezing storage of meat and produced their special effect at the favourable temperature and humidity. Contamination of meat with moulds generally originated from slaughter halls and surrounding environment. They are responsible for a major protein of food deterioration in developing countries. Their presence in meat is considered as an indicator of the hygienic conditions under which meat is produced and stored leading to either spoilage or food borne mycotoxicosis **[33]** .

Table (6) declared that the concentration of aflatoxin B_1 range from 1.1 to 53 µg/kg (ppb) and 1.5 to 5.6 µg/kg (ppb) with mean concentration of aflatoxin B_1 was (7.8±2.2 and 3.4±0.5) for frozen meat cuts and minced meat respectively. The data illustrated in table(6) for aflatoxin B_1 our results is to somewhat higher than reported by **[34]** who illustrated that aflatoxin B_1 in frozen meat samples was 4.80 ± 0.89 but in the same study aflatoxin B_1 in minced meat was 3.62 ±0.88 nearly similar to our results. The obtained result not agree with **[35]** who not detected Aflatoxin B_1 and G_1 in examined minced meat samples. While **[36]** detected aflatoxins residues in minced meat were detected in 19 (76%) minced meat. Concentration of aflatoxins residues (µg/Kg) in the examined samples were with a mean of 2.5 ± 0.07. According to the permissible limits stated by **[37]** permissible limits 15 ppb for aflatoxin B_1 concentration in meat.

The Food and Agriculture Organization (FAO) determined the maximal daily intake of aflatoxin $B_1$to be 5 µg/kg and the total aflatoxin (B1 + B2 + G1 +G2) to be 10 µg/kg. This toxin has been classified by **[6]** in carcinogenic effect Group 1.Consuming meat products contaminated with aflatoxin can cause mutagenic, carcinogenic and teratogenic effects in consumers. Regarding this issue, it is important to evaluate the researches which show us the aflatoxin contamination of meat and meat products.

Regarding the results tabulated in **table (7)** the ochratoxin could be detected in 6 samples of frozen meat cuts and 14 samples of frozen minced meat each constituting (12% and 28%), respectively. The minimum detected level of Ochratoxin A in frozen meat cuts and frozen minced meat were 1.3 ppb and 2.5ppb, while the maximum was 6.2 ppb and 23ppb with a mean value of 3.9±0.8 µg/kg and8.1±1.9, respectively. From

our results is much higher than that reported by [36] who reported that ochratoxin A ranged from 0.05 to 2 µg/kg (ppb) in minced meat. According to the permissible limits stated by **[37]** permissible limits 5 ppb Ochratoxin A concentration in meat. Ochratoxin A is a potent toxin, causes nephrotoxicity and hepatotoxicity in human and animals **[38].** Moreover, it has immunosuppressive, teratogenic and carcinogenic effects where it disturbs cellular physiology in multiple pathways. Generally the variation in mycotoxin concentration may be due to the initial fungal contamination load.

Mycotoxins are transported to humans by consumption of meat obtained from animals fed with contaminated feeds and contaminated spices used during processing of these meats. Mycotoxins are thought to be responsible for various pathological syndromes in humans. For example, ochratoxin is associated with Balkan endemic nephropathy and fumonisin B_1 is associated with esophagus cancer . Today, more than 300 mycotoxins are known. Aflatoxins, ochratoxin A (OTA), fumonisins, trichothecenes and zearalenone toxins are the groups of mycotoxins, which have the largest agricultural, economic and public health importance **[39].**

4. CONCLUSION AND RECOMMENDATION

To improve the public health and reduce the economic losses due to mould growth and mycotoxin production, the following measures should be conducted strict hygienic measures started from slaughter house should be applied, with daily washing and disinfection of the floor, walls and all other utensils with powerful fungicidal agents. Application and implementation of Hazard Analysis and Critical control point (HACCP) system may be the appropriate solution to ensure quality and safety of food.

Table (1): Statically analytical results of total mould and yeast count of examined meat samples (n=50)

Type of Samples	Positive samples		Count of CFU/g.		
	No.	%	Min.	Max.	mean± S.E.
Meat cuts	43	86	1x10	$5.4x10^4$	$4.5x10^3±1.3x10^3$
Minced meat	50	100	1x10	$4.1x10^5$	$2.8x10^4±9.6x10^3$

Table (2): Comparison between meat cuts and minced meat as regarding to total mould and yeast count

Count of C.F.U/g.	Meat cuts	Minced meat	t	P
mean± SD	$4.5x10^3 \pm 8.8x10^3$	$2.8x10^4 \pm 6.8x10^4$	0.02	< 0.05

Significant = P < 0.05

Table (3): Identification of mould species isolated from examined samples

Mould	Species	Meat cuts		Minced meat	
		No.	%	No.	%
Aspergillus	*A. flavus*	18	40	10	21.2
	A. niger	22	48.8	28	59.5
	A. terreus	5	11.1	3	6.4
	A. aculeatus	0	0	2	4.3
	A. fumigatus	0	0	4	8.5
Penicillium	*P. fellutanum*	5	27.7	4	14.3
	P. citreonigrum	2	11.1	7	25
	P. aurantigroseum	6	33.3	9	32.1
	P. thomii	5	27.7	8	28.5
Acremonium spp.	*A. strictum*	3	100	7	100
Alternaria spp.	*Al. alternate*	10	100	13	100
Chaetomonium spp.	*Ch. brasiliense*	0	0	4	100
Curvularia spp.	*C. lunata*	4	100	1	100
Endomyces spp.	*E. fibuliger*	2	100	0	0
Eupenicillium spp.	*Eup. javanicum*	3	100	0	0
Fusarium spp.	*F. verticillioides*	0	0	3	100

Table (4): Incidence of yeast species isolated from the examined samples

Sample / yeast species	Meat cuts		Minced meat	
	No.	%	No.	%
Candida sp.	15	50	31	48.4
Rhodotorula spp.	4	13.3	12	18.7
Saccharomyces spp.	5	16.6	8	12.5
Torulopsis spp.	6	20	10	15.6
Ustilaga spp.	0	0	3	4.7
Total	30		64	

Table (5): Identification of Candia and Rhodotorula species isolated from examined samples

Yeast	Species	Meat cuts		Minced meat	
		No.	%*	No.	%*
Candida spp.	*C. albicans*	1	6.6	2	6.5
	C. tropicalis	2	13.3	3	9.7
	C. guillermondii	7	46.6	11	35.5
	C. krusei	2	13.3	3	9.7
	C. valida	0	0	5	16.1
	C. parapsilosis	3	20	7	22.6
Rhodotorula spp.	*R. mucilaginosa*	4	100	10	83.3
	R. glutinis	0	0	2	16.7

*%= calculated according to total number of each species/sample

Table (6): Average concentration of aflatoxin B_1 µg/kg (ppb) in examined samples (n=50)

Samples	Aflatoxin B_1 µg/kg (ppb)				
	No.	%	Min.	Max.	Mean ± SE
Frozen meat cuts	23	46	1.1	53	7.8±2.2
Frozen minced meat	7	14	1.5	5.6	3.4±0.5

Table (7): Average concentration of ochratoxin A µg/kg (ppb) in examined samples (n=50).

Samples	Ochratoxin A				
	No.	%	Min.	Max.	Mean ± SE
Frozen meat cuts	6	12	1.3	6.2	3.9±0.8
Frozen minced meat	14	28	2.5	23	8.1±1.9

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