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MYCOLOGICAL ASPECT OF MEAT COLD STORE AT KALYOBIA GOVERNORATE

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A B S T R A C T

A random swabs from frozen meat, fish and chicken meat (total=45, n=15 of each), walls and air of cold stores (n=45 per each) in Kalyobia governorate were subjected to mycological examination and determination of the ability of aflatoxins production by isolated molds. The obtained results declared that the mean total mold counts in the examined swabs of meat, fish and chicken meat (cfu/cm^2) were 1.12±0.29×10^5, 5.81±0.97×10^4 & 9.37±2.65×10^3 for a cold store. The total mold count (cfu/cm^2) were varied from 3.0×10^3 to 5.4×10^5 with an average of 2.61±0.37×10^5 for the meat cold store walls, 3.0×10^3 to 2.0×10^5 with an average of 6.75±1.14×10^4 for fish cold store walls and 1.0×10^3 to 1.6×10^5 with an average of 2.58±0.49×10^4 for chicken meat cold store walls. Aspergillus, Cladosporium, Fusarium, Mucor, Nigrospora, Penicillium, Rhizopus, Sporotricum, Thamnidium and Tricoderma species were isolated and identified from the examined swabs of meat, fish and chicken meat as well as walls and air of cold store with different percentages. Also, genus Aspergillus (A.) was further identified as *A. flavus*, *A. fumigatus*, *A. nigar*, *A. ochraceus*, *A. terreus and A. vesicolor* were recovered from examined swabs with varying percentages. The average concentrations of aflatoxin B_1, B_2, G_1 and G_2 (µg/1) extracted from toxigenic strains of *A. flavus* isolated from examined swabs of cold store were 63.27±3.15%, 31.85±1.73, 35.78±1.98 & 18.62±1.03 for meat, 50.61±2.72, 22.48±1.19, 27.06±1.54 & 11.97±0.75 for fish and 37.46±1.95, 16.35±0.88, 21.70±1.10 & 8.64±0.59 for chicken meat, respectively. The public health significance of the isolated mold species and the probable sources of refrigerated meat with such serious organisms as well as some recommendation to prevent them to grow and/or produce their aflatoxins were discussed.

KEY WORDS: Aflatoxin, Cold store, Frozen meat, Health hazardous, Mycotic.

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1. INTRODUCTION

The continuously increasing demand for wholesome food has initiated the concerned authorities to import large quantities of meat, poultry and fish beside the local production of tease food materials. So, many cool stores have been constructed to organize this trade by offering a good deal of cold storage facilities.

Meat, poultry and fish are subjected to contamination with several types of microorganisms from different sources, during the period that elapses from the time of slaughtering or catching till consumption of such contaminants which may render the product as inferior quality or even unfit for consumption, thus resulting in economic losses and at times may constitute a public health hazard [8]. Meat contaminated with some molds may become spoiled or may be incriminated in human mycosis. Molds have been recorded as constitute a public health hazard because they produce mycotoxins.

It is interesting to study molds as an important origin of meat contamination. in spite of the non-pathogenically of most molds, it must be stressed that meat may

assume a moldy odor and taste if the affection is extensive and for long standing may aid in production of fat rancidity. As several molds could be isolated from surfaces of refrigerated meat, yet deep-freezing has no significant destructive effect among molds as Aspergillus (A.) species had received a great attention as it can produce aflatoxin, which has a great public health hazards.

Therefore, this work was planned out to study the hygienic status of a cold store considering the walls and air in Kalyobia Governorate and the stored food materials. Accordingly, all collected samples were exposed to 1. Determination of mold count for the cold stored meat, fish and chicken meat. 2. Determination of mold count in the swabs of cold store air and walls. 3. Isolation and identification of isolated mold species. 4. Determination of aflatoxins produced by aflatoxigenic fungi.

2. MATERIAL AND METHODS

2.1. *Collection of samples*

A total of forty five swabs of frozen meat, fish and chicken meat (30 of each) were collected from a cold store in Kalyobia governorate (n=15 per each food item). Moreover, 90 random swabs were taken from the walls and air of cold store (n=45 of each). All collected samples were subjected to mycological examination and determination of the ability of isolated molds for production of aflatoxins [2]. All collected samples were examined as quickly as possible to evaluate their mycological quality. Swabs were represented by sterile cotton screw capped plastic tubes which are ready for use. A template made of metal having an exposed inner area of 10cm^2 (2×5cm) was used to delineate area of sampling. The template were wrapped in aluminum foil and sterilized in hot air oven at 180°C for 20 minutes.

2.2. *Preparation of rinsing fluid*

Buffered peptone water 1% was used as rinsing and diluents fluid. The solution was distributed to small heat resistant screw capped tubes, each containing 10 ml of rinsing fluid, and then sterilized in the autoclave at 121°C for 20 minutes [11].

2.3. *Cold store air samples*

Sabaroud dextrose agar plates were placed inside the examined cold store at a distance of one meter above the floor, thus, such plates were opened for one minute to represent the air samples of cold stores according to the technique recommended [2].

2.4. *Swabbing of meat, fish and chicken surface and walls*

Swabs from meat, fish and chicken surface and walls of cold stores were taken after the use of sterile cotton swab and template. The sterilized template placed firmly against the surface to limit the examined area. The sterile cotton swabs drawn from screw capped plastic tubes , moistened in rinsing fluid solution (buffered peptone water 1%), then rolled over the limited area of carcass inside the template rolled in one direction and perpendicular to this direction to represent all area. Finally, cotton swabs were aseptically retained into the rinsing fluid screw capped tubes containing 10 ml buffered peptone water (1%). The collected swab samples transferred immediately to the laboratory without undue delay.

2.5. *Preparation of swabs*

The collected swabs were mixed in 90 ml of sterile buffered peptone water to give 1/10 dilution. However, one ml of the dilution was mixed with 9 ml of buffered peptone water in a test tube and the contents were mixed carefully, then ten-fold serial dilutions were prepared as formerly described [11].

2.6. *Determination of total mold count*

One ml of previously prepared serial dilution was aseptically transferred into

double sterile Petri dish, and then 10 ml of sabaroud dextrose agar media previously melted and cooled at 45℃, were added and thoroughly mixed. Moreover, the plates were left to solidify at room temperature then incubated at 25℃ for 7 days. During the incubation period the incubated plates were examined daily till the star-shape colonies appeared and total mold count/cm^2 was then calculated and recorded [2].

2.7. *Isolation and identification of mold*

It was carried out according to their morphological characters, mold colonies were picked up with their surrounding medium under aseptic conditions and transferred to sabaroud dextrose agar slopes and incubated at 25℃ for 7 days for further identification of genus Aspergillus [14], mold and other mold genera [20]. Qualitative and quantitative estimation of aflatoxins by thin layer chromatography was done as described by Shin and Marth [18]. Aflatoxins in samples extracted were separated and resolved on glass plates coated with silica gel. Developed plates were examined with the aid of ultraviolet light (365). Aflatoxins concentration was determined visually by comparing the intensities of fluorescence of spots in the sample with those of appropriate aflatoxin standard [2].

3. RESULTS AND Discussion

Mold spoilage of refrigerated foods may cause considerable economic loss through discoloration of these products because most mould genera can grow at low temperature [17]. Moreover, the toxic mold metabolites especially aflatoxins are broad spectrum active substances which produced as a result of the growth of certain molds on the various kinds of foods. Results given in the table 1 indicated that the total mold count (cfu/cm^2) were ranged from 2.7×10^3 to 5.0×15^5 with an average of $1.12\pm0.29\times10^5$ for meat, 1.0×10^3 to 2.2×10^5 with an average of $5.81\pm0.97\times10^4$ for fish and 9.0×10^2 to 8×10^4 with an average of 9.37×10^3 for chicken meat of cold store. However, the different mold genera were detected in the examined swabs of meat, fish and chicken with percentages of 93.33%, 86.67% and 80.00% for cold store. The differences associated with the examined samples of meat, fish and chicken meat were highly significant ($p< 0.01$) as a result of total mold count.

The current results were nearly similar to those reported by previous authors [5, 6, 9, 15].

Results achieved in table 2 recorded that the total mold counts (cfu/cm^2) were varied from 3.0×10^3 to 5.4×10^5 with an average of $2.61\pm0.37\times10^5$ for meat cold store walls, 3.0×10^3 to 2.0×10^5 with an average of $6.75\pm1.14\times10^4$ for fish cold store walls and 1.0×10^3 to 1.6×10^5 with an average of $2.58\pm0.49\times10^4$ for chicken meat cold store walls.

However, the percentage of mold genera isolated from the examined wall swabs of meat, fish and chicken meat were 100%, 86.67% and 86.67% for cold store.

It is evident from these results recorded in table 3 the total mold (cfu/cm^3) were ranged from 1.0×10^3 to 8.3×10^4 with an average of $2.27\pm0.43\times10^4$ for meat cold store air 5.0×10^2 to 6.2×10^4 with an average of $9.55\pm2.39\times10^3$ for fish cold store air and 2.0×10^2 to 1.1×10^4 with an average of $4.72\pm0.65\times10^3$ for chicken meat of cold store air. However, the mold genera were detected in the examined air samples were 86.67%, 80.00% and 80.00% for meat, fish and chicken meat cold store air. Generally, the contamination of the foods of the animal origin with mold may be originated from air, soil, utensils and wall of cold store as well as the poor hygienic measures adopted inside the cold store [3]. Concerning the quality of the stored food stuffs, molds con deteriorate such food through production of proteolytic and lipolytic enzymes [13].

Table 1 Total mould counts in the examined swabs of meat, fish and chicken meat at a cold store (n=15 per each).

	Positive samples		Min.	Max.	Mean± S.E
	No.	%			
Meat	14	93.33	2.7×10^3	5.0×10^5	$1.12\pm0.29\text{x}10^5$
Fish	13	86.67	1.0×10^3	2.2×10^5	$5.81\pm0.97\text{x}10^4$
Chicken Meat	12	80.00	9.0×10^2	8.0×10^4	$9.37\pm2.65\text{x}10^3$

Table 2 Total mould counts in the examined swabs of cold store walls (n=15 per each).

Food item	Positive samples		Min.	Max.	Mean± S.E
	No.	%			
Meat	15	100	3.0×10^3	5.4×10^5	$2.61\pm0.37\times10^5$
Fish	13	86.67	3.0×10^3	2.0×10^5	$6.75\pm1.14\times10^4$
Chicken Meat	13	86.67	1.0×10^3	1.6×10^5	$2.58\pm0.49\times10^4$

Table 3 Total mould counts in the examined swabs of cold store air (n=15).

Food item	Positive samples		Min.	Max.	Mean± S.E
	No.	%			
Meat	13	86.67	1.0×10^3	8.3×10^4	$2.27\pm0.43\times10^4$
Fish	12	80.00	5.0×10^2	6.2×10^4	$9.55\pm2.39\times10^3$
Chicken Meat	12	80.00	2.0×10^2	1.1×10^4	$4.72\pm0.65\times10^3$

Data in table 4 reveled that Asperagillus species (46.67%) were the most fungal species isolated from the examined meat samples of the cold store, followed by Penicillium (40.00%) and Fusarium species (26.67%). Concerning the cool store, Penicillum, Asperagellus and Cladosporium were isolated from the examined fish swabs at percentages of 46.67%, 40.00% and 26.67%, respectively. Furthermore, the mold species isolated from chicken meat samples of the cold store were Asperagellus and Penicillum with the same percentages (26.67%) followed by Rhizopus (20.00%).

The cold stores are the most common source of the mold on stored meat where Penicillium (blue green mold), Cladosporium species (black spots), Rhizopus, Mucor, Thammidium (Wisker) and Crysporium (white spots) can be seen over the surface of refrigerated meat [7]. The most important form of spoilage was found to be black spots, which caused by *Cladosporium herbarium* in examined frozen meat samples kept at -5.5°C [16]. Penicillium species were the main molds isolated from refrigerated food (meat, fish and chicken meat) due to its ability to grow in adverse condition and the ability of many species of Penicillium to grow on refrigeration temperature less than -2℃ [19].

Table (4): Incidence of mould species isolated from the examined swabs of meat, fish and chicken meat at a cold store (n=15).

Mould species	Meat		Fish		Chicken meat	
	No.	%	No.	%	No.	%
Aspergillus	7	46.67	6	40.00	4	26.67
Cladosporium	3	20.00	4	26.67	2	13.33
Fusarium	4	26.67	2	13.33	1	6.67
Mucor	3	20.00	3	20.00	2	13.33
Nigrospora	1	6.67	-	-	-	-
Penicillium	6	40.00	7	46.67	4	26.67
Rhizopus	2	13.33	1	6.67	3	20.00
Sporotricum	-	-	2	13.33	-	-
Thamnidium	2	13.33	3	20.00	1	6.67
Trichoderma	-	-	-	-	1	6.67

The results achieved in table 5 declared that the incidence of Aspergilli isolated from the examined swabs of meat of the cold store were the *A. flavus* (26.67%), *A. nigar* (13.33%) and *A. vesicolor* (6.67%). Concerning the swabs isolated from fish of the cold store, *A. flavus* (26.67%), *A. fumigatus* (6.67%) and *A.*

vesicolor (6.67%) were isolated and identified. In case of examined swabs of chicken meat of cold store while, the isolation percentages of *A. flavus*, *A. ochraceus* and *A. terreus* were 13.33%, 6.67% and 6.67%, respectively. The most important factors influencing growth and aflatoxin production by *A. flavus* are the moisture content of the substrate and the relative humidity of the environment. In this respect, *A. flavus* cannot invade substrate blow 17.5% moisture [10].

Table 5 Incidence of Aspergillus species isolated from the examined swabs of meat, fish and chicken meat at a cold store (n=15).

Aspergillus species	Meat		Fish		Chicken meat	
	No.	%	No.	%	No.	%
A. flavus	4	26.67	4	26.67	2	13.33
A .fumigatus	-	-	1	6.67	-	-
A. niger	2	13.33	-	-	-	-
A.ochraceus	-	-	-	-	1	6.67
A. terreus	-	-	-	-	1	6.67
A. vesicolor	1	6.67	1	6.67	-	-
Total	7	46.67	6	40.00	4	26.67

Table 6 indicated that the incidence of aflatoxigenic strains of *A. flavus* isolated from examined swabs of meat, fish and chicken meat in the cold store were 20.00%, 13.33% and 13.33%, respectively. Aflatoxins are the most important mycotoxins produced by *A. flavus* which can result in acute liver cirrhosis, carcinogenic, mutagenic and teratogenic effects on consumers of contaminated food items containing these toxic substances. The production of aflatoxin by *A. flavus* was controlled by oxygen and sodium chloride requirements which increase the mold growth and enhance the production of aflatoxin [4].

Approximately, 50.00%of *A. flavus* and *A. parasiticus* strains were toxigenic. In addition, the moisture content of the food above 15% supports the growth of these mould and aflatoxin elaboration [12].

It is obvious from the results recorded in table 7 that the type and average levels of aflatoxins B_1, B_2, G_1 and G_2 (µg/1) extracted from toxigenic strains of *A. flavus* isolated from the examined swabs of the cold store were 63.27±3.15, 31.85±1.73, 35.78±1.98 &18.62±1.03 for meat, 50.61±2.72, 22.48±1.19, 27.06±1.54 & 11.97±0.75 for fish and 37.46±1.95, 16.35±0.88, 21.70±1.10 and 8.64± 0.59 for chicken meat, respectively.

It is worth mentioned that Aflatoxin B_1 is the most potent carcinogen even at very low concentrations as compared with other types of aflatoxins [1].

Table 6 Incidence of mould species isolated from the examined swabs of meat, fish and chicken meat at a cold store (n=15 per each).

Type of sample	Type of *A. flavus*			
	Toxigenic		Non-toxigenic	
	No.	%	No.	%
Meat	3	20.00	1	6.67
Fish	2	13.33	2	13.33
Chicken meat	2	13.33	-	-

Table 7 Types and levels of aflatoxins (µg/L) extracted from the toxigenic strains of *A. flavus* isolated from the examined swabs of meat, fish and chicken meat (n=15).

Aflatoxin Species	Meat	Fish	Chicken meat
B_1	63.27±3.15	50.61±2.72	37.46±1.95
G_1	35.78±1.98	27.06±1.54	21.70±1.10
G_2	18.62±1.03	11.97±0.75	8.64±0.59

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الجوانب الفطرية لمبردات اللحوم فى محافظة القليوبية

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الملخص العربي

تمثل مبردات الأغذية دورا حيويا في الحفاظ على جودة و صلاحية الأغذية ذات الأصل الحيواني وخصوصا اللحوم والأسماك و الدواجن . وعلى الجانب الأخر , تمثل الفطريات تحديا كبيرا فى تلوث هده الاغذيه عندما لا يتم تطبيق الاشتراطات الصحية السليمة للمحافظة على نظافة تلك المبردات. لذلك تم جمع 45 مسحه من أسطح الحوم، الأسماك والدواجن (15 من كل نوع) المحفوظة داخل مبردات للحوم بمحافظة القليوبية. علاوة على اخذ 90 مسحة من جدران و هواء المبردات وذلك لتحديد مدى تلوثها بالفطريات المختلفة. دلت نتائج الدراسة علي أن متوسط العدد الكلى للفطريات (ميكروب/سم^2) فى مسحات أسطح اللحوم، الأسماك والدواجن كان $1.12\pm0.29\times10^5$، $5.81\pm0.97\times10^4$ و $9.37\pm2.65\times10^3$ للمبرد. كان العدد الكلى للفطريات (ميكروب/سم^2) يتراوح من 0.3×10^3 إلى 5.4×10^5 بمتوسط $2.61\pm0.37\times10^5$ للجدران المحيطة باللحوم و من 3×10^3 إلى 2×10^5 بمتوسط $6.75\pm1.14\times10^4$ للجدران المحيطة بالأسماك و من 1×10^3 إلى 1.6×10^5 بمتوسط $2.58\pm0.49\times10^4$ للجدران المحيطة بالدواجن. علاوة على ذلك كان متوسط العدد الكلي للفطريات (ميكروب/سم^2) لعينات الهواء للمبرد المحيطة بكل من اللحوم $2.27\pm0.43\times10^4$ و $9.0\pm2.39\times10^3$ للأسماك و 4.72×10^3 للدواجن. تم عزل العترات التالية: الاسبراجلس، كلادوسبورم، فيوزريم، ميوكر، نيجروسبورم، بنسليم، ريزوبس، سبوروتريكم، ثمانيديم و تريكودرما، وأيضا التعرف عليهم من المسحات المأخوذة من اللحوم والأسماك والدواجن و من الجدران المحيطة والهواء للمبرد بالنسب التالية: 46.67، 40، و26.67 %. كما تم أيضا تصنيف عتر الاسبراجلس من المسحات بنسب مختلفة على النحو التالى: اسبراجلس فلافس، اسبراجلس فيوميجاتس، اسبراجلس نيجر، اسبراجلس اكراسيس، اسبراجلس تيريس، و اسبراجلس فيزيكولر.

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