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Incidence of lipolytic and proteolytic fungi in some chicken meat products and their public health significance

***Shaltout, F. A.; **El-diasty, E.M. and ***Mohamed, M. S.**

*Faculty of Vet. Med., Moshtohr, Benha University

** Animal Health Research Institute Dokki, Giza

***Egypt food factories

ABSTRACT

A total of 100 random samples of chicken meat products; 25 of chicken fillet, chicken luncheon, chicken pane and chicken minced meat; were collected and subjected to mycological evaluation. The mean total mould counts were $1.9x10^2 \pm 8.2x10^1$, $3.3\ x10^2 \pm 2.0\ x\ 10^2$, $2.8x10^2 \pm 1.4\ x\ 10^2$ and $1.9\ x10^2 \pm 3.4x10^1$ CFU / g for chicken fillet, chicken luncheon, chicken pane and chicken minced meat respectively. Respective yeast counts were $5.7x10^2 \pm 3.7x10^2$, $3.3\ x10^2 \pm 2.0\ x10^2$, $2.1\ x10^2 \pm 1.1\ x10^2$ and $4.3\ x10^3 \pm 9.8\ x10^2$CUF/ g. The predominant mould genera isolated from chicken fillet, chicken luncheon, chicken pane and chicken minced meat were Aspergillus followed by Penicillium then Geotrichum, Fusarium; Cladosporium; Mucor; Eupencillium; Scopulariopsis and Acremonium. The predominant species of yeasts isolated from chicken fillet, chicken luncheon, chicken pane and chicken minced meat were Candida spp. (32.5%) followed by Rhodotorula spp. (22.1%), Saccharomyces spp. (18.2%), Torulopsis spp. (15.5%) and Cryptococcus spp. (11.7%). The isolated moulds and yeasts were evaluated for proteolytic and lipolytic activities on skim milk agar and Tributyrin agar. The economic and public health significance of isolated moulds and yeasts as well as the sanitary precautions were discussed.

INTRODUCTION

Chicken and chicken products provide a source of animal protein of high biological value for consumers at all ages, where they contain all the essential amino acids required for growth with high proportion of unsaturated fatty acids and low cholesterol contents. Moreover, poultry meat is good source of different types of vitamins as niacin, riboflavin, thiamine and ascorbic acid as well as sodium, calcium, iron, phosphorus, sulpher and iodine**.**

In processing plants, contamination of poultry meat products can occur throughout ideal processing, packaging and storage until the product is sufficiently cooked and consumed. Heavy microbial loads enter the processing operations with the

living birds and these microorganisms can be disseminated throughout the plant during processing. Diseases can also result when these products are not properly cooked and post-processing contaminated (**Zhang *et al*., 2001)**.

Fungi are of considerably less importance in poultry spoilage except when antibiotics are employed to suppress bacterial growth. When antibiotics are employed, however, moulds become the primary agents of spoilage. The genera of Candida, Rhodotorula Debaryomyces, and Yarrowia are the most important yeasts found on poultry meat (**Jay, 1979).**

However, moulds and yeasts are of great importance in spoilage of poultry meat products resulting in different changes in flavour, colour, texture and odour and also these fungi responsible for major portion of food deterioration especially in poor developing countries due to lacke of hygienic measure and due to the use of contaminated additives and spices which considered a major important sources of mould contamination **(Abd El-Rahman, 1987)**.

Therefore, the present study was planned out to throw a light on the total mould and yeast counts of chicken products (pane, minced meat and luncheon), and determination of lipolytic and proteolytic activities of the existing moulds and yeast.

MATERIALS AND METHODS

Collection of samples

A total of 100 random samples of processed chicken meat products (25 samples of each chicken fillet, pane, minced meat and luncheon) were collected from shops and supermarkets. These samples were obtained and preserved in an ice box, then transferred to the laboratory under complete aseptic conditions and examined as rapidly as possible.

Fungal isolation and identification

Total fungal count was carried out according to the techniques recommended by **ISO (217-1-2:2008).** Fungi were isolated and identified according to macroscopic and microscopic characteristics as described by **Pitt and Hocking (2009)**. Identifications of the yeast isolates were performed according to **Kriger Van Rij (1984) and Deàk, 2008)**.

***Evaluation of lipolytic and proteolytic activities of existing moulds and yeasts isolated from examined samples*:**

Lipolytic activity was determined by using Tributyrin agar medium according to the technique recommended by **Koburger and Jacger (1987)**. Proteolytic activity was determined by using a casein substrate as described by **O'reilly and Day (1983).**

RESULTS

Table (1): Incidence of moulds and yeasts in examined chicken meat products (N= 25).

Examined samples	Chicken Fillet		Chicken Luncheon		Chicken Panne		Chicken Minced meat	
	No. of positive samples	%	No. of positive samples	%	No.of positive samples	%	No.of positive samples	%
Mould	15	60	16	64	25	100	20	80
yeast	21	84	20	80	17	68	25	100

Table (2): Statistical analytical results of total mould count/g of examined chicken meat products samples.

Examined samples	Minimum	Maximum	Mean ± SE.
Chicken Fillet	10	$2x10^3$	$1.9x10^2 \pm 8.2x10^1$
Chicken luncheon	20	$4x10^3$	$3.3x10^2 \pm 2.0x10^2$
Chicken Pane	20	$3.5x10^3$	$2.8x10^2 \pm 1.4x10^2$
Chicken Minced meat	20	$8.1x10^3$	$1.9x10^2 \pm 3.4x10^1$

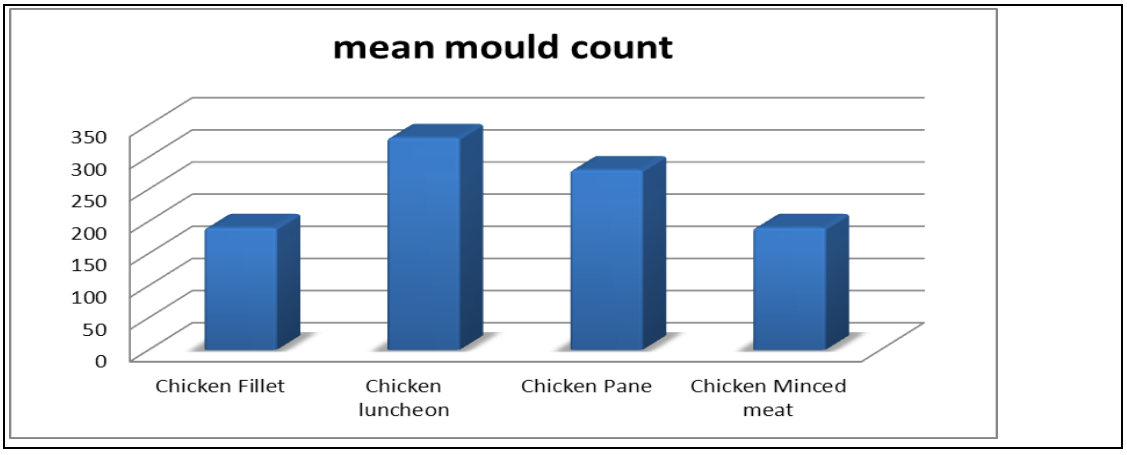

Figure (1): Mean mould counts / g of examined chicken meat product samples

Table (3): Statistical analytical results of total yeasts count/g of examined chicken meat products samples. (N=25)

Examined samples	Minimum	Maximum	Mean ± SE.
Chicken Fillet	20	$2.9x10^3$	$5.7x10^2 \pm 3.7x10^2$
Chicken luncheon	50	$2.9x10^3$	$3.3x10^2 \pm 2.0x10^2$
Chicken Pane	<10	$2.6x10^3$	$2.1x10^2 \pm 1.1x10^2$
Chicken Minced meat	$1.2 x10^2$	$1.7x10^4$	$4.3x10^3 \pm 9.8x10^2$

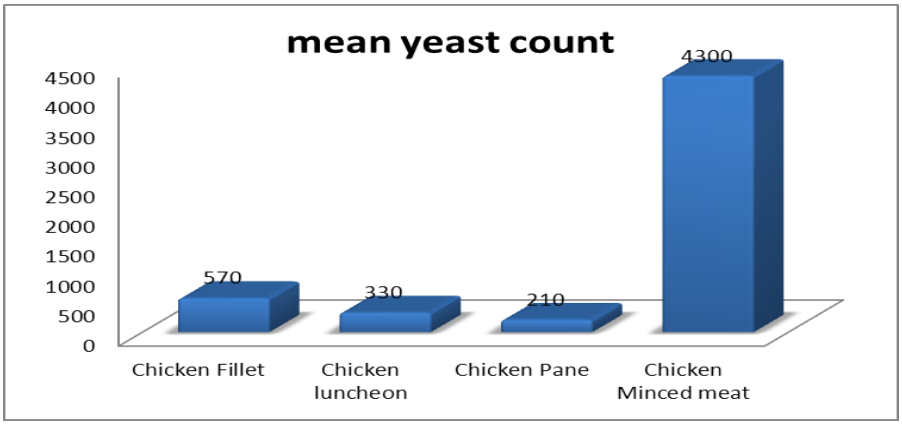

Figure (2): Mean yeast counts / g of examine d chicken meat product samples

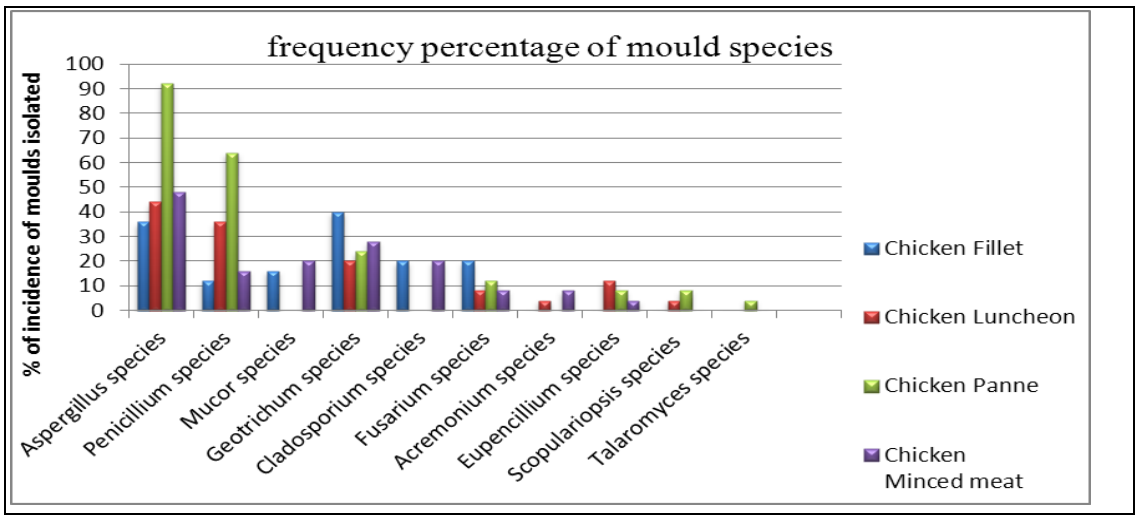

Figure (3): Frequency percentage of moulds species isolated from examined samples of chicken meat products.

Table (4): Incidence of Aspergillus species isolated from examined samples of different chicken meat products (N=55).

Product / Aspergillus species	Chicken Fillet		Chicken Luncheon		Chicken Pane		Chicken Minced meat	
	No.	%	No.	%	No.	%	No.	%
A. flavus	3	5.5	5	9.1	7	12.8	6	10.9
A.niger	5	9.1	6	10.9	8	14.5	6	10.9
A. ochraceus	-	-	-	-	5	9.1	-	-
A. terreus	-	-	-	-	2	3.6	-	-
A. clavatus	-	-	-	-	1	1.8	-	-
A .candidas	1	1.8	-	-	-	-	-	-

N= Number of isolated Aspergillus species

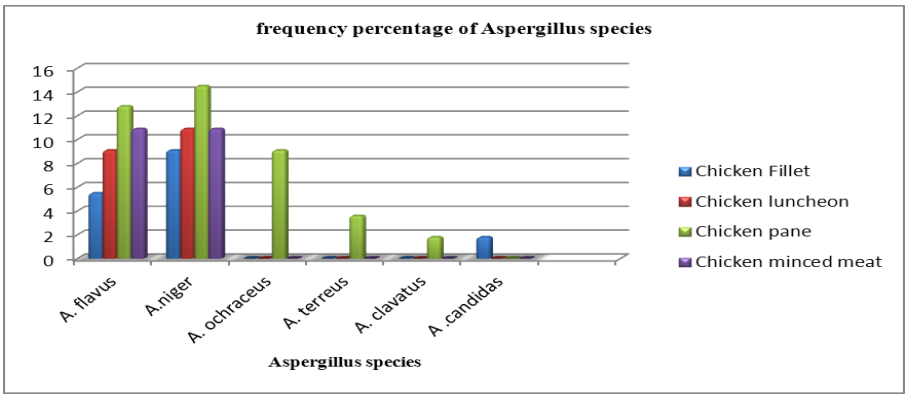

Figure (4): Frequency percentage of Aspergillus species isolated from examined chicken meat product (according to number of isolated Aspergillus species)

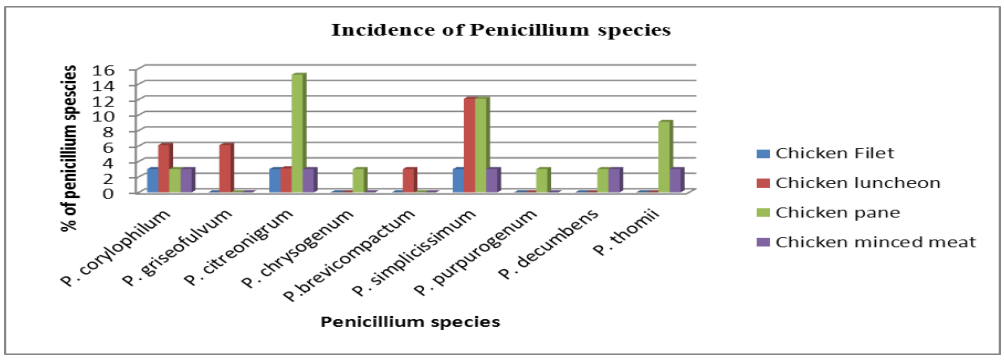

Figure (5): Frequency percentage of Penicillium species isolated from examined chicken meat product (according to number of isolated Penicillium species)

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Table (5): Incidence of yeast species isolated from examined samples of chicken meat products (N=79).

Products / yeast species	Chicken Fillet		Chicken Luncheon		Chicken Pane		Chicken Minced meat	
	No.	%	No.	%	No.	%	No.	%
Candida spp.								
C. albicans	-	-	-	-	-	-	1	1.3
C .famata	-	-	1	1.3	1	1.3	1	1.3
C. .krusei	3	3.8	1	1.3	1	1.3	1	1.3
C. .lusitaniae	-	-	1	1.3	-	-	1	1.3
C. parapsilosis	2	2.6	1	1.3	1	1.3	2	2.6
C. pelliculosa	1	1.3	1	1.3	-	-	1	1.3
C. tropicals	-	-	1	1.3	1	2.6	1	1.3
Cryptococcus spp.	-	-	-	-	1	2.6	3	3.8
Cry. albidus	-	-	1	1.3	1	1.3	-	-
Cry. species	-	1.3	2	2.6	1	1.3	3	3.8
Saccharomyces spp.								
S. cerevisiae	1	1.3	-	-	1	1.3	2	2.6
S. species	2	2.6	5	6.4	1	1.3	2	2.6
Rhodotorula spp.								
Rho. glutinis	1	1.3	2	2.6	2	2.6	2	2.6
Rho. species	3	3.8	2	2.6	1	1.3	4	5.1
Torulopsis spp.	2	2.6	3	3.8	2	2.6	5	6.4

N= Number of isolated yeast species

Table (6): Proteolytic and lipolytic activity of some isolated mould.

Mould species	Proteolytic activity	Lipolytic activity
A. flavus	+ve	+ve
A .candidas	+ve	+ve
A. clavatus	-ve	-ve
A. ochraceus	+ve	+ve
A.niger	+ve	+ve
A. terreus	+ve	+ve
P. brevicompactum	-ve	+ve
P. citreonigrum	+ve	+ve
P. chrysogenum	+ve	+ve
P. corylophilum	+ve	+ve
P. decumbens	-ve	+ve
P. griseofulvum	+ve	+ve
P. purpurogenum	+ve	+ve
P. simplicissimum	-ve	+ve
P. thomii	+ve	+ve

Table (7): Proteolytic and lipolytic activity of some isolated yeasts.

Yeast species	**Proteolytic activity**	**Lipolytic activity**
C. albicans	-ve	+ve
C .famata	-ve	+ve
C. .krusei	+ve	+ve
C. .lusitaniae	+ve	+ve
C. parapsilosis	+ve	+ve
C. pelliculosa	+ve	+ve
C. tropicals	+ve	+ve
Cry. albidus	-ve	+ve
S. cerevisiae	-ve	+ve
Rho. glutinis	-ve	+ve

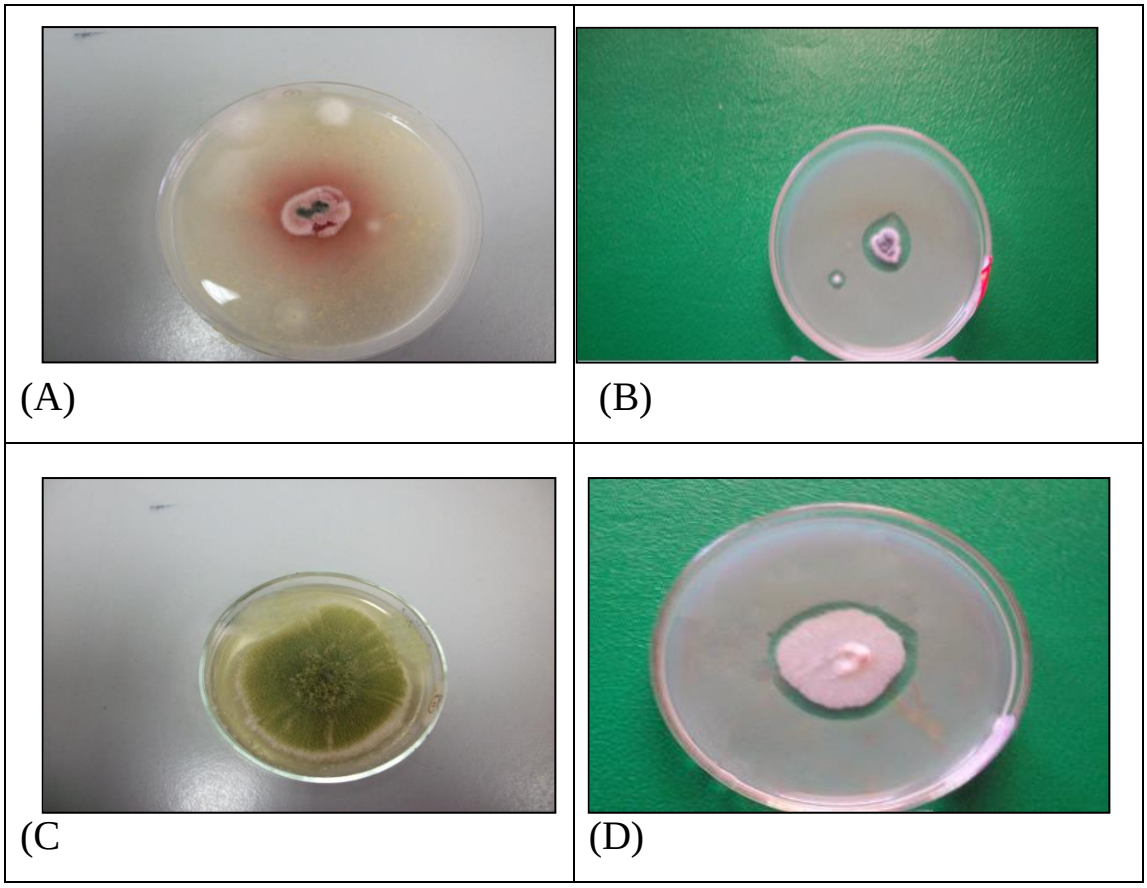

Figure (6): Show proteolytic and lipolytic activity of isolated moulds and yeasts, (A) Proteolytic activity of *Penicillium purpurogenum*,(B) Lipolytic activity of *Penicillium griseofulvum*,(C) Lipolytic activity of *A. flavus* and (D) Lipolytic activity of *C. krusei*

DISCUSSION

Moulds only compete with bacteria on meat when storage temperatures are lowered to 0^0C or below, or when the meat surface dries to an a_w that enables fungi to compete **(Pitt and Hocking, 2009).**

Several authors cited several reports of bacteria and fungi growing on meats at -5^0C, with yeasts and moulds predominating as temperatures were further lowered, to a limit at about -12^0C. **Schmidt-Lorenz and Gutschmidt (1969)** reported that moulds and yeasts grew on chickens stored at –7.5 and -10 ± 0.2^0C for 1 year.

The result achieved in table (1) revealed that the incidence of moulds in the examined chicken meat product samples were 15(60%), 16 (64%), 25(100%) and 20(%80) for chicken filet, chicken luncheon, chicken pane and chicken minced meat respectively. While, the incidence of yeasts in the examined chicken meat product samples were 21(84%), 20 (80%), 17(68%) and 25(%100) for chicken filet, chicken

luncheon, chicken pane and chicken minced meat respectively. The results obtained for chicken filet, chicken luncheon, chicken pane and chicken minced meat are similar to that recorded by many investigators **(Abdel-Rahman *et al*., 1985, Edris 1986 and Rwaih-Wadee, 2010)** who stated that the incidence of moulds in the examined chicken meat product samples were 13 (86.6%), 14 (93.33%), 13 (86.6%) of chicken luncheon, chicken pane and chicken minced meat respectively.

From the results achieved in table (2) and figure (1) it is evident that the mean mould count in chicken filet was 1.9 $x10^2$ ±8.2 $x10^1$ cfu/g, while chicken luncheon was 3.3 $x10^2$± 2.0 x 10^2 cfu/g. Concerning the examined samples of chicken pane and chicken minced meat, it is evident from the results presented in figure (1) that the mean values of mould count 2.8$x10^2$ ±1.4 x 10^2 and 1.9 $x10^2$ ± 3.4$x10^1$ cfu / g, respectively.

The obtained results declared that the examined chicken luncheon samples had the highest mould count followed by chicken pane samples, while the chicken minced meat had the lowest count.

The obtained results were higher than those reported by **Rwaih-wadee (2010)** who found that the mean value of total fungal counts in examined samples of chicken luncheon, chicken pane and chicken minced meat were 58.86 ± 14.44, 44.60 ± 13.28 and 53.33 ±21.25 cfu/g. While higher figures were revealed by **Shaltout (1996), El-Deeb et al. (2011), Shawish (2011), Sharaf and Sabra (2012), and Yousif *et al*. (2013), El-diasty *et al*. (2013)** who reported that the mean values of the total mould count of the chicken meat slice, chicken luncheon and chicken minced meat were 2.51, 3.71 and 4.12 log CFU/g, respectively.

Regarding the results recorded in table (3) and figure (2) it is obvious that the mean yeast count of examined samples in chicken filet, chicken luncheon, chicken pane and chicken minced meat were 5.7 $x10^2$± 3.7 $x10^{2,}$ 3.3 $x10^2$± 2.0 $x10^2$, 2.1 $x10^2$± 1.1 $x10^2$ and 4.3 $x10^3$ ± 9.8 $x10^2$CUF/ g, respectively. The minimum counts were of 20, 50, <10 and 1.2$x10^2$, respectively. While, the maximum count were 2.9$x10^3$, 2.9 $x10^3$, 2.6 $x10^3$ and 1.7 $x10^4$, respectively. Higher counts were obtained by **Brr and Mahmoud (2005), Hassan (2007), Ouf *et al*. (2010), El-Deeb *et al*. (2011)** and **El-diasty *et al*., (2013).** Comparatively most of the previous researches had higher counts due to the differences between the chicken processing plant at this time

(sanitation program – computerized machines – educated workers) and manufacturing in the past.

Chicken Luncheon and Chicken pane is exposed to heat treatment during its processing and any microbial activities may be recorded as a result of post processing contamination, unhygienic storage as well as long storage life which may lead to spoilage. Also, spices have been used in many meat industries and this may add more contamination to meat products. Having been dried material from plant origin, spices are commonly heavily contaminated with xerophilic storage moulds. The most frequent fungal contaminants of spices are species from the genera Aspergillus and Penicillium (**Kocic´ -Tanackov *et al*., 2007)**.

The results given in figure (3) revealed that the chicken meat products samples were contaminated with many fungal genera and species. The predominant mould genera isolated from chicken filet, chicken luncheon, chicken pane and chicken minced meat were Aspergillus 9(36%), 11(44%), 23 (92%) and 12(48%) respectively, followed by Penicillium 3(12%), 9(36%), 16 (64%), and 4(16%) then Geotrichum 10 (40%), 5(20%), 6(24%) and 7(28%);Fusarium 5 (20%), 2(8%), 3(12%) and 2(8%); Cladosporium 5 (20%), 0 (0%), 0 (0%) and 5 (20%); Mucor 4 (16%), 0 (0%), 0 (0%) and 5 (20%); Eupencillium 0 (0%), 3(12%), 2(8%) and 1(4%) ; Scopulariopsis 0 (0%), 1(4%), 2(8%) and 0 (0%); Acremonium 0 (0%), 1(4%),0 (0%) and 2(8%) Finally Talaromyces isolated only from Chicken Pane 1(4%). Nearly similar strains were recorded in different poultry meat products by **Abdel-Rahman *et al*., (1985), Edris (1986), El-Khateib and Abd El-Rahman (1989), Edris *et al*. (1992), El-Gazzar (1995), Shaltout (1996), Shaltout (2002), Bkheet *et al.* (2007), Hussein (2008), Shawish (2011) and El-Diasty *et al.* (2013).**

The present data in table (4) and figure (4) shows the number and percentages of Aspergillus isolated from examined samples, *A. niger* was 5 (9.1%), 6 (13.04%), 8 (17.4%) and 6 (13.04%) from Chicken Fillet, chicken luncheon, chicken pane and chicken minced meat samples respectively, followed by *A. flavus* 5 (5.5%), 5 (10.9%), 7 (15.21%) and 6(13.04%) respectively, *A. ochraceus, A .terreus* and *A. clavatus* detected in chicken pane only 5 (10.9%), 2(4.34%) and 1 (2.2 %) respectively. *A. candidas* 1 (1.8%) isolated only from Chicken Fillet.

The obtained results declared that the chicken pane and chicken minced meat samples had the highest number of Aspergillus spp. 23 and 12 isolates, respectively, followed by chicken luncheon (11 isolates). Aspergillus is a uniquitous soil–dwelling fungus **(Wong *et al.* 2008).**

Identifications of Penicillium spp. isolated from the examined chicken meat products in figure (5) revealed that *P. citreonigrum, P. simplicissimum* were the most predominant species isolated from the chicken luncheon, chicken pane and chicken minced meat samples, 1(3.3 %), 5(16.7%) and 1(3.3 %), respectively, and 4(13.3%), 4(13.3%) and 1(3.3 %), respectively. *Penicillium corylophilum* 1 (3.0%), 2(6.7%), 1(3.3 %) and 1(3.3 %), respectively in chicken fillet chicken, luncheon, chicken pane and chicken minced meat samples. *Penicillium thomii* and *P. decumbens* were 3(10%) and 1(3.3 %), respectively, and 1(3.3 %) and 1(3.3%), respectively in chicken pane and chicken minced meat samples only. In chicken luncheon *P. griseofulvum* and *P. brevicompactum* 2(6.7%) and 1(3.3 %), respectively, *P. chrysogenum* 1(3.3 %) in chicken pane and *P. purpurogenum* were isolated from chicken pane 1(3.3 %).

Some fungi may produce secondary metabolites known as mycotoxins. Mycotoxins are potent animal carcinogens and have been classified by the International Agency for Research in Cancer **(IARC, 1993)** as human carcinogens or potential (probable and possible) human carcinogens.

Regarding the results tabulated in table (5) shows the incidence of yeast genera isolated from the examined samples of chicken filet, chicken luncheon, chicken pane and chicken minced meat Candida spp. 25 (32.5%) was the predominant yeast species isolated from such samples followed by Rhodotorula spp. 17(22.1%), Saccharomyces spp. 14 (18.2%), Torulopsis spp. 12 (15.5%) and Cryptococcus spp. 9 (11.7%).

Nearly similar results were recorded by **Edris (1992)** ,**Hegazi *et al*. (1992), Shaltout (1996), Viljoena *et al*. (1998), Shaltout (2002), Brr and Mahmoud (2005), Deák (2008), Ouf *et al*. (2010), Shawish (2011), Seham- Ismail (2013), El-Diasty et al. (2013)** and **Samaha (2013) .**

Moulds and yeasts are capable of hydrolyzing a wide range of proteinaceous materials **(Koburger, 1972)**. In this respect, the proteolytic activity of isolated moulds from examined chicken meat products was evaluated using litmus milk medium

(Table, 6 and 7). The obtained results showed that *A. flavus, A.niger, A. ochraceus, A. terreus, A. candidus, P. corylophilum, P. griseofulvum, P. Citreonigrum, P. chrysogenum, P. simplicissimum, P. purpurogenum, P. thomii*, were having proteolytic activity with clear zone of casein hydrolysis. While, all Candida species had proteolytic activity except *C. albicans*, also *C. krusei, Cry. albidus*, S. *cerevisiae and Rho. glutinis*.

On the other hand the lipolytic activity revealed that nearly all the isolated fungi and species of *C. albicans, C. krusei, C. parapsilosis, C. pelliculosa, C .lusitaniae, C. tropical, C .famata, Cry. albidus, S. cerevisiae* and *Rho. glutinis* were found to be lipolytic on the tributyrin agar media. These results nearly similar to those obtained by **Abd El-Rahman and Saad (1989), Welthagen and Viljoen (1999), Abd El-Aziz (1999), Farag (2000), Nasser (2002), Shaltout (2002), Subash, *et al*.(2005), Soliman and Shalaby (2008), Korashy and Wahbba (2008), El-Diasty and Salem (2009)** and **Ouf *et al*. (2010).**

In conclusion, the obtained results showed high contamination of chicken meat products with different types of yeasts and moulds which constitute a public health hazard. Obviously, it is important to prevent mould growth to avoid toxin production through preventing the natural contamination of raw materials. Storage of food under conditions which prevent mould growth and strict hygienic measures and regulations should be imposed during processing, packing and transportation.

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الملخص العربي

مدي تواجد الفطريات المحللة للبروتين والدهون في بعض منتجات لحوم الدجاج وأهميتها الصحية

*فهيم عزيزالدين شلتوت و **ايمان محمود الدياسطي و ***محمد سامي محمد

*كلية الطب البيطري بمشتهر- جامعة بنها
** قسم الفطريات بمعهد بحوث صحة الحيوان بالدقي
*** مصانع اجيبت فود

تم فى هذه الدراسة تجميع عدد 100 عينة بطريقة عشوائية من منتجات لحوم الدواجن بواقع 25 عينة من كل من فيلية الدجاج و لانشون الدجاج والبانيه ولحم الدجاج المفروم من الأسواق المختلفة . وقد تم فحص هذه العينات لتقييمها من حيث تعرضها للتلوث بالفطريات المختلفة . وقد وجد أن متوسط العدد الكلى للأعفان بتلك العينات هو كالتالى (٩ ,١ x١٠ ٢±٢ ,٨ x١٠ ١ ; ٣,٣ x١٠ ٢ ±٠ ,٢ x١٠ ٢ ; ٨ ,٢ x١٠ ١ ±٤ ,١ x١٠ ٢ و٩ ,١ x١٠ ٢±٤ ,٣ x١٠ ١ مستعمرة / جرام) لكل من عينات فيلية الدجاج و لانشون الدجاج والبانيه ولحم الدجاج المفروم على الترتيب . أما بالنسبة للعد الكلى للخمائر فقد كان ٧,٥ x ١٠ ٢ ± ٣,٧ x ١٠ ٢; ٣ ,٣ x١٠ ٢ ±٠ ,٢ x١٠ ٢ ; ١ ,٢ x ١٠ ٢ ± ١,١ x١٠ ٢ و ٣ , ٤ x ١٠ ٣ ± ٨ , ٩ x١٠ ٢ مستعمرة / جرام) . وقد كانت أكثر الأعفان التى تم عزلها من العينات هى فطر الأسبرجيليس يليه البنسيليوم ثم الجيوتركيم ، الفيوزاريوم ، الكلادسبوريم ،الميوكور ، الأيوبنسيليوم، الأسكوبيولاريوبسيس وأخيرا الأكرمنيوم . وبالنسبة للخمائر الأكثر عزلا من عينات منتجات لحوم الدواجن فقد كانت الكانديدا (٥ ,٣٢ %) يتبعها الرودوتيرلا (١ , ٢٢ %) ، السكارروميسيس (٢ , ١٨ %) ، التيريولبسيس (٥ , ١٥ %) وأخيرا خمائر الكربتوكوكس (٧ , ١١ %) . تم تقييم المعزولات من الأعفان والخمائر محل الدراسة لنشاطها الأنزيمى المحلل للبروتين والدهون على البيئات الخاصة بهذا الغرض. وقد تم مناقشة الأهمية الأقتصادية والصحية للفطريات التى تم عزلها الى جانب مناقشة الأحتياطات الصحية لتجنب مشاكل تلك الفطريات.