

Hygienic significance of fungal flora of locally produced chicken and turkey fillet with trials of control by using some herbs.

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Abstract

A total of 50 samples of fillet represented by chicken and turkey (25 of each) were collected randomly from retail markets and shops in Cairo and Giza governorate and the collected samples subjected to sensory and mycological examination. The obtained results indicated that mean value of molds count and yeast count of chicken fillet were 2.0 ± 0.04 , 2.2 ± 0.04 , 2.9 ± 0.03 , 3.0 ± 0.03 , 2.9 ± 0.03 , 3.1 ± 0.03 , 4.1 ± 0.02 , and 4.0 ± 0.02 log cfu/g. While the mean value of molds count and yeast count of turkey fillet were 1.4 ± 0.08 , 1.4 ± 0.08 , 2.7 ± 0.06 , 2.9 ± 0.06 , 1.6 ± 0.08 , 2.0 ± 0.09 , 2.5 ± 0.04 , and 2.7 ± 0.04 log cfu/g at 0, 3, 7, 10 days respectively. The total mean of mold and yeast count in chicken and turkey fillets were time dependent, where, the population of fungal contamination elevated by increase the time of storage from 0-10 days. These results revealed that long period of storage, increase fungal count. We can isolate *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium expansum*, *Penicillium verrucosum*, *Candida albicans* and *Rhodotrula* spp. Most of isolated mold and yeast from fillet samples showed proteolytic and lipolytic activity. The lipolytic activities of some tested fungi were reached to 100%. The antifungal activity of garlic and black pepper was evaluated by their direct addition to meat fillet samples and were found to have the highest desirable effects.

Keywords: Fungal flora, hygienic significance, chicken and turkey fillet, herbs.

Introduction

The progressive increase in meat consumption becomes a worldwide problem associated with the rapid increase in human population particularly in developing countries as Egypt. Chicken meat is an important food item in most countries due to its contribution in solving the problem of animal meat shortage. Poultry meat is a suitable meal for feeding infants, young children, adult and convalescents as well as a good supplement to a deficient diet.

Chicken and chicken meat products are good sources of animal protein of high biological value, which contains all the essential amino acids required for human nutrition, besides that they contain higher proportion of unsaturated fatty acids and less cholesterol especially when skin is removed (Shedeed, 1999). The acceptance of further processed chicken meat prod-

ucts depends upon overall acceptance, color, odor, taste and consistency. So, consumers had given much greater choice over the foods which are more selective of high quality and cheap about the value of money. Finally, the product quality became more significant factor in meat products marketing (Agamy and Hegazi, 2001).

However, the quality of poultry meats is detected and observed by its appearance and texture. Appearance of meat is critical for both the consumers's initial selection of the product as well as for final product satisfaction. While the texture is the single most important sensory property affecting final quality assessment. So, since appearance is so critical for consumer selection, producers continuously aimed to go to great length produce products with the appropriate color for products market and to

avoid appearance defects, which negatively affect product selection or price. So, color and texture are necessary to produce consistently high quality poultry products. (**Fletcher, 2002**).

On the other hand, the evaluation of human food of animal origin for fungal contamination is urgently required for updating measurement the foods quality and the degree of its deterioration, which are essential components for the microbiological assurance programs. (**Marta et al., 2001**).

However, the molds and yeasts deterioration of food caused high economical losses in these products. They may grow on the surfaces of meat and meat products causing sliminess, off odors, off tastes, and discoloration due to white, creamy, pink and brown pigments and become unfit for consumption (**Fraizer & West Hoff, 1978**).

Several environmental factors associated with breeding of food producing animal are the main sources of fungal contamination of meat included the surrounding air, soil of poultry breeding and rearing houses, old litter, litter-containing water, wet feed and bird droppings (**Bryan, 1980**).

Whereas, other factors of fungal contamination of food related to the environment of slaughtering as contaminated feathers, feed and the carcasses of the slaughtered birds have been found to be contaminated with molds and yeasts (**Barnes, 1976**). Molds and yeasts contamination of such food resulted significant economical losses in poultry production and may lead to spoilage (**Viljoen et al., 1998**). Moreover, yeast affections of meat may cause change the flavors and diminishing the quality of meat products that rendering them unmarketable and unfit for human consumption (**Jay, 1978**). Also, (**Walker, 1977**) mentioned that several species of fungi lead to undesirable changes in foods, particularly the yeasts species which able to cause significant lipolytic and proteolytic changes in foods even if present in low counts in meat.

Hence, the preservation of food free from con-

taminants gained the attention of all food authorities and the problem of food preservation has grown in complexity. As the frequent food products introduction on the markets, the requirement for longer shelf life and greater assurance of protection from microbial spoilage were undertaken (**Farag et al., 1989**).

The most safe and valuable methods to achieve the quality assurance of meat and their products for human consumption were the addition of some herbs during their processing. The antimicrobial activity of garlic, cinnamon and cloves has been studied since the end of the last century (**El-gayyar et al., 2001**).

Black pepper has demonstrated impressive antioxidant and antimicrobial effects-yet another way in which this wonderful seasoning promotes the health of the digestive tract. And not only does black pepper help you derive the most benefit from your food, the outer layer of the peppercorn stimulates the breakdown of fat cells, keeping you slim while giving you energy to burn (**Dorman and Deans, 2000**).

Therefore, this work was designed to study of organoleptic examination of chicken and turkey fillet, mold and yeast count, isolation and identification of mold and yeast. In addition, some herbs were used for inhibition the fungal contamination of meat as the garlic and pepper herbs.

Materials and Methods

Collection of samples: A total of fifty chicken and turkey fillet (25 of each) were collected from different Cairo and Giza markets and then subjected to the following examination:

Organoleptic examination: it was carried to evaluate color, flavor and texture as described by **Smith et al., (1973)**. A score 1 is being disliked extremely and 10 being like extremely.

samples preparation: were carried out according to **ISO (6887-2003)**

Mycological examination: Determination of molds and yeasts count: according to **ISO (21527-1/2008)**

Isolation and Identification of molds and yeast from samples: Ten grams of each sample were

transferred aseptically into sterile blender jar, to which 90 ml of 1% peptone water were added and homogenized in a sterile warring blender for 2 minute, and tenfold serial dilutions of the homogenate were prepared (ICMSF, 1978). One milliliter quantities of the previously prepared serial dilutions were inoculated separately into Petri dish plates and mixed with Sabouraud dextrose agar medium supplemented with chloramphenicol 0.05 mg/ ml. The plates were then left to solidify after mixing and incubated at 25°C for 3-5 days. The counts of mould colonies were recorded. Individual suspected colonies were selected depending upon their morphological characters. Stock culture were made from each isolate and monitored on Czapek-Dox, malt extract, potato dextrose (PDA) agar slopes and rice agar plates for further identification. The identification of mould and yeast species was carried out by observation of macroscopic and microscopic characteristics of mold and yeast colonies according to Pitt and Hocking (2009).

Proteolytic and lipolytic activities of molds and yeasts isolated from the examined chicken and turkey fillet: Some of the isolated mold and yeast from the present samples were screened for their proteolytic and lipolytic activities.

Proteolytic activity of isolated fungi:

A casein substrate was used according to

O'reilly and Day (1983). Either of isolated mould or yeast was inoculated on the surface of skim milk agar plates and was incubated at 25°C for 7 days. The clear zones of hydrolysis mean positive results (degradation of protein around the fungal colony).

Lipolytic activity of isolated fungi: Tributyrin agar medium was used according to Koburger and Jacger (1987). Isolated mould and yeast were separately inoculated onto the surface of tributyrin agar plates, to which 0.05 mg chloramphenicol per ml was added. The inoculated plated were incubated at 25°C for 7 days. The clear zones of hydrolysis surrounded lipolytic colonies but medium appeared opaque.

Evaluation the effect of garlic and black pepper on the growth of fungi in chicken fillet samples (Yadav *et al.*, 2002 and Ali and Flora 2015): One kilogram from examined chicken fillet samples were divided into slices each one weight 50 gram and mixed with 2% garlic powder and 0.5% black pepper. The treated fillet samples were chilled for 10 days. Then examined for mold and yeast count at 0, 3rd, 7th and 10th days.

Statistical Analyses: The obtained data were analyzed for significance, calculation of standard error according to SAS/STAT (2000).

Results and Discussion

Table (1). Mean value of organoleptic examination of fresh chicken and turkey fillet at zero, 3rd and 7th days

	Chicken fillet			Turkey fillet		
	Color	Flavor	Texture	Color	Flavor	Texture
Zero day	7.4 ± 0.2	7.6 ± 0.13	8.2 ± 0.25	8.6 ± 0.14	8.35± 0.22	9.2±0.29
3rd day	6.9±0.2	7.1±0.13	7.7± 0.25	8.1±0.15	7.85±0.22	8.6±0.28
7th day	5.4± 0.2	5.6±0.13	6.2± 0.25	6.6±0.15	6.35±0.22	7.2±0.3

Table (2). Total mold and yeast counts of fresh refrigerated chicken fillet (log cfu/g)

State of colony count	Total fungal colony count in samples							
	O day		3 days		7 days		10 days	
	Mould	Yeast	Mould	Yeast	Mould	Yeast	Mould	Yeast
Minimum	1.0	2.0	1.0	2.0	2.0	3.1	2.0	3.3
Maximum	3.7	4.8	3.7	4.7	3.9	5.3	4.0	5.6
Mean	2.0	2.9	2.2	3.1	2.9	4.1	3.0	4.0
SE	0.04	0.03	0.04	0.03	0.03	0.02	0.03	0.02

Table (3). Total mold and yeast counts of fresh refrigerated turkey fillet (log cfu/g).

State of colony count	Total fungal colony count in samples							
	Zero day		3 days		7 days		10 days	
	Mould	Yeast	Mould	Yeast	Mould	Yeast	Mould	Yeast
Minimum	1.0	1.0	1.0	1.0	2.0	2.0	2.0	2.1
Maximum	3.0	2.8	3.0	3.0	3.5	3.0	3.7	3.2
Mean	1.4	1.6	1.4	2.0	2.7	2.5	2.9	2.7
SE	0.08	0.08	0.08	0.09	0.06	0.04	0.06	0.04

Table (4). Prevalence of mold and yeast in chicken fillet and turkey fillet.

Fungal species	Chicken fillet (25)		Turkey fillet (25)	
	No. +ve	%	No. +ve	%
<i>A. flavus</i>	10	40	-	-
<i>A. niger</i>	8	32	8	32
<i>P. chrysogenum</i>	21	84	4	16
<i>P. expansum</i>	12	48	6	24
<i>P. verrucosum</i>	4	16	-	-
<i>C. albicans</i>	24	96	6	24
<i>Rhodotrula</i>	10	40	2	8
<i>Cladosporium.</i>	8	32	2	8

%; was calculated according to number of the examined samples

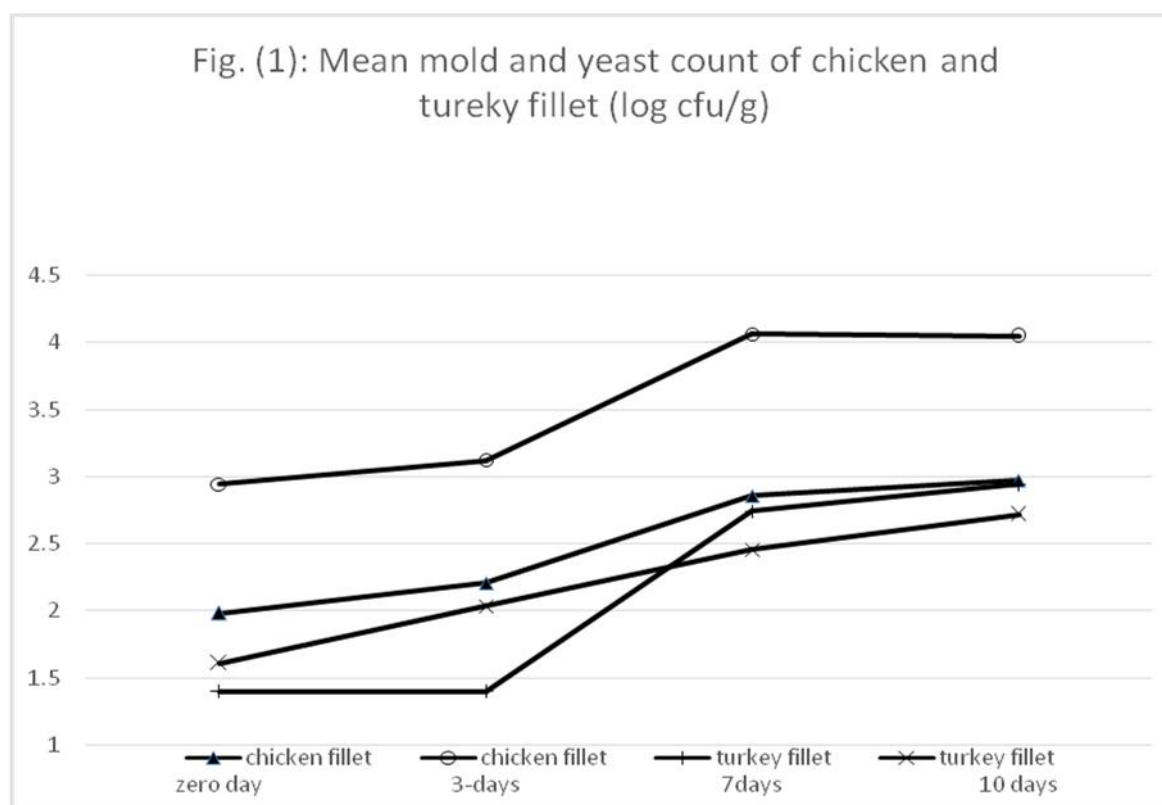


Table (5). Proteolytic activity of some isolated molds from the examined chicken and turkey fillet samples on litmus milk medium.

Molds and Yeast	Chicken fillet			Turkey fillet		
	No. of tested isolates	Positive isolates		No. of tested isolates	Positive isolates	
		No.	%		No.	%
<i>A. flavus</i>	10	10	100	0	0	0.00
<i>A. niger</i>	10	10	100	2	2	100
<i>P. chrysogenum</i>	4	4	100	1	1	100
<i>P. expansum</i>	5	5	100	2	2	100
<i>P. verrucosum</i>	2	2	100	0	0	0.00
<i>c. albicans</i>	20	20	100	4	4	100
<i>Rhodotrula</i>	3	3	100	0	0	0.00

Table (6). Lipolytic activity of isolated molds and yeast from the examined chicken fillet and turkey fillet samples on tributynin medium.

Molds and Yeast	Chicken fillet			Turkey fillet		
	No. of tested isolates	Positive isolates		No. of tested isolates	Positive isolates	
		No.	%		No.	%
<i>A. flavus</i>	10	10	100	0	0	0.00
<i>A. niger</i>	10	7	70	2	1	50
<i>P. chrysogenum</i>	4	3	75	1	1	100
<i>P. expansum</i>	5	4	80	2	1	50
<i>P. verrucosum</i>	2	1	50	0	0	0.00
<i>c. albicans</i>	20	20	100	4	2	50
<i>Rhodotrula</i>	3	3	100	0	0	0.00

Table (7). Effect of antifungal activity of mixed 2% garlic and 0.5% black pepper on fungal contamination of chicken fillet .

	Zero day	3 th day	7 th day	10 th day
Total fungal colony count	5.0X 10 ³	2.0 X 10 ²	1.0 X 10	0.0

The progressive increase in consumption of poultry meat and their products gained the attention of all authorities in Egypt to raise the hygienic quality of consumed meat as guarantee of human health. Fungi are unable to grow rapidly on animal tissue. However, when meat preservation techniques such drying, curing, aging and freezing are employed, fungi can compete effectively with bacteria and become dominant microflora (Pestka, 1986).

Appropriate color, flavor and texture of marketed chicken fillet are necessary to produce consistently high quality poultry products (Fletcher, 2002).

Data obtained from Table (1) revealed that

mean value of color, texture, and flavor of chicken fillet at zero day, were 7.4±0.2, 7.6±0.13 and 8.2±0.25 respectively. While at third day the result where 6.9±0.2, 7.1±0.13 and 7.7 ± 0.25. At the seventh day of examination the results where 5.4 ± 0.2, 5.6± 0.13 and 6.2±0.25 respectively, also from the same table the color, flavor and texture of turkey fillet at zero day were 8.6±0.14, 8.35± 0.22 and 9.2±0.29 but at the third day of examination the results were 8.1±0.15, 7.85±0.22 and 8.6 ±0.28 respectively. At the seventh day of examination the results were 6.6±0.15, 6.35±0.22 and 7.2±0.3 respectively, it's obvious that the results of the organoleptic examination of tur-

key fillet were better than the chicken fillet, this results also indicate that the results of zero day and third day was nearly similar but it changed at the seventh day.

At the tenth day of examination the examined samples were deteriorated.

Organoleptic examination such as appearance, taste, aroma, and texture of chicken and turkey meat can generally produce a consumer's decision to purchase type of meat. Flavor comprises mainly taste and aroma and involves in consumers' meat purchasing behavior and preferences even before the meat is eaten (**Shahidi, 1989, Sitz et al., 2005**).

Yeasts and Molds counts in meat and meat products were used as a sanitary index for the quality of meat and processed meat products (**Hefnawy, 1978**).

Moreover, yeast affections lead to certain defects that may change the flavors and quality of meat products rendering them unmarketable or inappropriate for human consumption (**Jay 1978 and Yadav et al., 2002**). Whenever, the absence of hygienic measures adopted during preparation, processing and storage of food leads to growth of moulds and consequently produced mycotoxines which are carcinogenic, teratogenic, nephrogenic and immunosuppressive metabolites for human consumption (**Refai and Hassan, 2013**).

Currently, the results in (Table, 2) showed that the mean value of mold count in chicken fillet were 2.0 ± 0.4 , 2.2 ± 0.04 , 2.9 ± 0.03 , 3.0 ± 0.03 . Whereas, the mean value of yeast count in chicken fillet were 2.9 ± 0.03 , 3.1 ± 0.03 , 4.1 ± 0.02 , 4.0 ± 0.02 , at 0, 3, 7, and 10 days times of examination respectively. The obtained results were relatively lower than that reported by **Abdel Aziz et al., (2005)** and **Patricia et al., (2006)** who recovered the mean mould and yeast count at the range of 10^3 - 10^5 . The Egyptian standardization of poultry meat mentioned that the surface of chicken carcasses must be free from any fungal growth and didn't mentioned that the count of mold and yeast which recorded at the first day of the examination. In this study macroscopic examination

revealed that no fungal growth on fillet surface.

On the other hand, the mean value of mold in turkey fillet was 1.4 ± 0.08 , 1.4 ± 0.08 , 2.7 ± 0.06 and 2.9 ± 0.06 , respectively. While, the mean value of yeast count in turkey fillet were 1.6 ± 0.08 , 2.0 ± 0.09 , 2.5 ± 0.04 and 2.7 ± 0.04 , at 0, 3rd, 7th, and 10th days respectively (Table, 3). These results were nearly similar to that obtained by **El-Daly and Neveen (2004)**. The lower counts were recorded by **Saleh et al., (1990)**. While the higher count were detected by **Hegazi et al., (1992)** and **Abdel Rahman et al., (1984)**. These variations may be attributed to the differences in hygienic condition during handling and processing of chicken meat and its products.

Regarding the prevalence of different species of fungi in chicken and turkey fillets, *C. albicans* and *P. chrysogenum* were the most predominant isolated from chicken fillet samples. While *A. niger* and *C. albicans* were at the top of all fungi that recovered from turkey fillets. The species of *A. flavus* was isolated from 40% of chicken fillet sample, but not present in any samples of turkey fillet (Table, 4)

These results were come in accordance with the results recovered by (**Saleh et al., 1990; Morshdy and Roushdy, 1992; Wafaa, 1995; Wafia and Hassan, 2000**).

Moreover, fungi are known to be enzymatically active organisms and the enzymes proteases and lipases are of particular interest in meat, as they cause degradation of proteins and fats leading to spoilage (**Smith and Hass, 1992**). The invading organisms may find the opportunity to grow and multiply in the product inducing undesirable changes, rendering the product unremarkable. Some species of the isolated moulds (*Penicillium*) have a lipolytic activity and were found to be causative agent of rancidity in food products. **Hassan et al. (2012)** reported that the majority of fungi were producer for proteases and the most proteolytic fungi were *A. niger* (100%) and *A. flavus* and *Penicillium* spp. (83.3%), followed by *A. ochraceus* (80%). Whereas, other fungi

showed lower proteolytic activity as *Candida albicans* (50%). Moulds and yeasts are capable of hydrolyzing a wide range of pertinacious materials (**Koburger, 1972**). Also, **Lotfi, et al., (1983)** stated that diatomaceous fungi on the surface of raw meat and milk products have proteolysis action and moldy odor. **Sayid (1999)** and **Wafia and Hassan, 2000** detected that most fungal isolates from meat products showed different degrees of proteolysis activities.

In the present work, the results presented in Table (5) indicated that the most prevalent and predominant mold and yeast isolated from chicken and turkey fillet were *A. flavus*, *A. niger*, *P. chrysogenum*, *P. expansum*, *P. verrucosum*, *C. albicans*, *Rhodotrula*. All these isolates showed proteolytic activity at the rates of 100%.

Regarding the lipolytic activity of tested fungi from the examined chicken fillet samples were reached to 100% by the isolates of *A. flavus*, *C. albicans* and *Rhodotrula* spp. While the lower lipolytic activity was observed by *P. verrucosum* that recovered from chicken fillet (50%) (Table 6). Whereas, the recovered *P. chrysogenum* from turkey fillet showed a maximum rate of lipolytic activity (100%), but *A. flavus*, *P. verrucosum* and *Rhodotrula* spp. not showed any lipolytic activity (0%) (Table 6).

These results were supported by **Godfredsen (1990)**, who reported lipolytic microorganisms such as *A. niger*, *A. flavus*, *A. fumigatus* and *Aspergillus* spp. in meat and fish meat and milk products. Also, **Smith and Haas (1992)** stated that, *Rhizopus*, *Geotrichum*, *Aspergillus*, *Mucor*, *Penicillium* and yeasts *Candida* and *Rhodotrula* showed significant lipolytic activity. Also, Yeasts were found to have a lipolytic activity and were used as a guide for proper sanitation and quality control of food products (**Ottogalli and Galli, 1972**). **Sayid (1999)** and **Hassan and Abdel Dyem (2004)** detected that most fungal isolates from meat products showed different degrees of proteolytic activities.

Several studies detected that the fungi constitute a public health hazard, as some members are incriminated in cases of pulmonary infection, urinary tract infection, vaginitis, thrush, arthritis, osteomyelitis, dermatitis, endocarditis, meningitis and eye infections (**Mossel, 1982**). *Aspergillus* species may induce pulmonary aspergillosis, allergy, skin infection, sinusitis as well as nail and ear infection (external otitis). Also some species of genus *Penicillium* may induce also pulmonary infection, external otomycosis mycotic ceratitis and endocarditis.

Other study by **Refai et al., (2014)** briefly illustrated the fungal infection and yeasts in different species of animal and human as a result of feeding infected food and feed with particular references to their diagnosis and recent treatment.

Therefore, the control of fungal contamination of food products becomes a critical demand. The food industry relies heavily upon the use of antimicrobial agents such as garlic and pepper for the extension of shelf life and the preservation of the freshness of products. Because of governmental restrictions and consumer demands, it is desirable to optimize the use of antimicrobials in foods (**Davidson and Parish 1989**). Some studies used garlic as a folk medicine since ancient times for a variety of ills including snakebite, hemorrhoids, rheumatism, abdominal pains and parasitic infections (**Moore and Atkins 1977**). Whereas, **Hassan and El Sharnoby (1997)** reported that the natural plant extracts as (garlic, onion, citrus and pepper) were be the most useful inhibitors of microbial growth of food and no side accumulation effects on the internal organs of human and animal in comparison with the use of chemical preservatives.

The present study evaluated the use of 2% garlic and 0.5% black pepper in elimination the fungal pollution in chicken fillets. As illustrated in (Table7), the antifungal activity of garlic and black pepper on polluted samples revealed that the total colony of fungi were decreased when increase the time of chilling as complete inhibition of fungal growth at the

tenth day. The total fungal colony counts were 5×10^3 at zero day, 2×10^2 at third day, 1×10^1 at seventh day and 0.0 at tenth day.

These results show that garlic and black pepper had highly antifungal effects which nearly similar with those recorded by **Yin and Cheng (1998)**; **Hassan and Mansour (2003)** and **Hassan et al. (2007)**.

Therefore perpetual investigations are continuously undertaken to find new methods and technology on a scientific basis to save these products from being spoiled on the markets. The sanitary measures in slaughter houses, transport vehicles and processing plants should be strictly imposed together with educational programs to producers and processors in order to improve the quality of these products (**Refai et al., 1993**).

Recommendation

The high incidence of fungi in food and the significant lipolytic and proteolytic effects of most isolates in present study warrant that there is urgent need to undertake fungal pollution awareness creation programs among different foods in Egypt.

Good quality raw chicken meat must be used in manufacture, furthermore, strict hygienic measures must be taken during slaughtering of chicken and the subsequent processing, handling and storage. Moreover, educational programs and training courses should be recommended to meat handlers and workers. In addition to the sanitary rules including personal hygiene, cleaning and disinfections of utensils and equipment's should be adopted with periodical checks of the meat products.

Food additives such as spices and herbs to avoid this contamination.

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الأهمية الصحية للفطريات بفيليه الدواجن و الرومي المنتجة محلياً ومحاولة السيطرة عليها بأستخدام بعض الأعشاب

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المخلص العربي

تم تجميع 50 عينة من فيليه الدواجن والديك الرومي (20 لكل منهما) عشوائياً من أسواق التجزئة والمحلات التجارية من كل من محافظة القاهرة والجيزة وأنهم تعرضوا للفحص الحسي والفطري. وكانت النتائج التي تم الحصول عليها هي: متوسط عد الفطريات و الخمائر في فيليه الدواجن 2.2 ± 0.04 ، 2.9 ± 0.03 ، 3.1 ± 0.03 ، 4.1 ± 0.02 و 4.0 ± 0.02 log cfu/g، بينما كان متوسط عد الفطريات و الخمائر في فيليه الديك الرومي 1.4 ± 0.08 ، 1.6 ± 0.08 ، 2.7 ± 0.06 log cfu/g وذلك في اليوم الأول والثالث رابع والعاشر على التوالي وأوضحت هذه النتائج انه كلما زادت مدة الحفظ زاد العد الفطري، حيث يرجع اسباب التلوث الفطري المرتفع إلى زيادة وقت تخزين 10-10 أيام. لقد تم عزل كل من فطر الاسبرجيللس فلافس و الاسبرجيللس نيجر، البنسليوم كريسوجينم، البنسليوم اكسبنزم، البنسليوم فيركوزم، كانديدا البيكانز و الرودوتريلا. أظهرت معظم الفطريات و الخمائر المعزولة من عينات الفيلية نشاط اذابة البروتينات والدهون. كما انه في هذه الدراسة نحاول السيطرة على نمو الفطريات عن طريق استخدام بعض الأعشاب مثل (الثوم والفلفل الأسود). كما تم تقييم النشاط المضاد من الثوم والفلفل الأسود بالاضافة المباشرة لعينات الفيلية ووجد أن لها أعلى التأثيرات المرغوبة.