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PROTEOLYTIC AND LIPOLYTIC ACTIVITY OF FUNGI ISOLATED FROM LUNCHEON MEAT AND POULTRY IN ASSIUT CITY

(With 3 Tables)

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النشاط الفطرى المحلل للبروتين والدهون للفطريات المعزولة من لانشون اللحوم والدجاج فى مدينة أسيوط

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لتقييم الحالة الميكولوجية للانشون (الدجاج واللحوم) تم جمع ٤٠ عينة عشوائية من مدينة أسيوط (٢٠ عينة لكل نوع) وقد تم العد باستخدام طريقة الزرع على الوسط الغذائى داى كلوران روز بنجال آجار والتحضين عند درجة٢٨°م لمدة تتراوح من ٥-٧ أيام وقد أظهرت النتائج أن كل العينات المستخدمة كانت عالية التلوث بالفطريات حيث تم التعرف على عدد ٣٥ فصيلة تابعة لـ ١٤ جنس وكان أكثرها شيوعا فصيلة الاسبريجلس نيجر, فلاقس وبارازيتكس التابعة لجنس الاسبريجلس ثم تلاها فى الظهور فصيلة البنسليوم كروزوجينم, كلوروفيلم التابع لجنس البنسليوم ثم فصيلة الترناريا اولترناتا و الميوكور سيدبنسيلويدس بينما كان الاسبرجلس فيوميجيتس و ميليس وتمارى والبنسليوم ستريتم أقلها شيوعا. وقد تم اختيار ٥٤ عزلة تابعة لـ ٢٦ فصيلة للتعرف على مقدرتها على افراز انزيمى الليباز والبروتييز وقد أظهرت النتائج أن النسبة كانت ٨١,٥ % , ٧٢,٢% من العترات المستخدمة لها القدرة على إفراز الإنزيمين على التوالى. وقد تمت مناقشة الأهمية الصحية والاقتصادية للفطريات والطرق المتبعة لمنع تلوث المنتج.

SUMMARY

Fourty samples of luncheon meat and poultry (20 samples for each) were collected from different supermarkets at Assiut City for mycological investigation. The plating technique using dichloran ros-bengal agar medium which incubated at 28°C was used for enumeration and isolation of fungi. The results indicated that all samples were highly contaminated with moulds. Some 35 species belonging to 14 genera were isolated. The most frequently encountered fungi were *Aspergillus*

niger, A. flavus, A. parasiticus. Penicillium chrysogenum, P.corylophilum, Alternaria alternata and *Mucor ciricinelloides. A. fumigatus, A. melleus, A. tamarii, P. citrinum, P.italicum* and *Scopuloriopsis brevicaulis* were less common. A total of 54 isolates, belonging to 26 species were tested for their abilities to produce lipase and protease enzymes. Of these isolates 81.5% and 72.2% could produce lipase and protease enzymes, respectively. The public health significance of isolated fungi was discussed.

***Key word:** Proteolytic and lypolytic activity of fungi isolted from luncheon*

INTRODUCTION

Meat and poultry products are valuable sources of protein but they are also an important potential source of serious disease if contaminated by different moulds which widely distributed in nature. These fungi are extremely considered as a major factor in the spoilage of meat products leading to great economic losses and constitute a major public health hazard by production of a wide variety of mycotoxins causing food poisoning and have carcinogenic effect in human (Mossel, 1982 and Foster,*et al,* 1983).

Luncheon may be contaminated by fungi either before and /or during processing, transportation and storage (El-Gendy and Morth, 1980 and Stoloff, 1984). Mould can cause deterioration of meat and meat products through production of proteolytic and lipolytic enzymes leading to discolouration, poor appearance , off-odour and off flavours (Koburger and Marth 1984, Besanco *et al.*,1992 and Jakobsen and Narvhus 1996).

Protease enzyme is produced by keratinolytic and non keratinolytic fungi but most active species were dermatophytes particularly species of Chrysosporium (Abdel-Gawad, 1997).

Lipase enzymes are generally quite stable and may retain activity in food for long periods even at low temperature..(Smith and Alford, 1984).

Many investigations concerned with studying lipase and proteolytic activities of many fungi (Barakat and Abdel-Sater, 1999; Aalbaek *et al.*, 2002; Abbas, *et al.*, 2002; Cabaleiro *et al.*, 2002; Germano *et al.*,2003 and Singh, 2003).

The purpose of this investigation was designed to study

1- The distribution and occurrence of fungi contaminating 40 luncheon samples (20 luncheon meat and 20 luncheon poultry)
2- Screening of the fungal isolates strains for their capabilities for production of protease and lipase enzyme.

MATERIAL and METHODS

-Collection of samples:

Fourty samples of luncheon meat and poultry (20 for each) were collected from different supermarkets in Assiut City. The samples were placed in a sterile plastic bags and transferred to the laboratory and kept at 4°C until fungal analysis.

Enumeration and isolation of fungi:

The direct plating technique (Pitt and Hocking, 1985) was employed for isolation of fungi from luncheon meat and poultry. Twelve pieces of luncheon of each sample (1x1 cm) were put on the surface of three plates of dichloran rose-bengal agar medium as reported by King *et al.*, (1979).

The plates were incubated at 28°C for 7 days and the growing fungi were counted, isolated and calculated per 12 pieces for each sample. Identification of fungi were based on macro and microscopic feature according to Raper and Fennel (1965), Pitt (1979); Domsch *et al.*,(1980); Kozakiewicz (1989); Moubasher (1993); Samson *et al.*,(1995) and Pitt and Hocking (1997).

-Screening for enzyme production:

A total of 54 fungal isolates representing 26 species and 11 genera isolated from luncheon meat and poultry were tested for their ability of producing lipase and protease enzymes.

-Lipase production:

The isolates were inoculated into deep slant of the basal medium as reported by Ullman and Blasins (1974). Positive results were recorded according to Hankin and Anagnostakis (1975). The lypolytic activity indicated by opaque zones surrounding microbial growth consisted of calcium salts of fatty acids.

-Protease production:

Proteolytic activity of selected moulds was detected using the medium reported by Ong and Gaucher (1973). The degree of enzyme activity was referred as weak, medium or high

RESULTS and DISCUSSION

The results revealed that all examined luncheon samples (100%) were contaminated with moulds, where the total count was 373 and 214/240 pieces of luncheon meat and poultry respectively (Table 1). A total of thirty five species belonging to 13 and 10 genera were isolated. The mean, standard deviation, minimum and maximum numbers of isolates of most common fungi from luncheon meat and poultry are presented in Table (2). The most prevalent genera in the two types of luncheon (Table 1) were *Aspergillus* and *Penicillium* followed by Mucor. These observations were not relatively agree with those indicated by Hamdy *et al.*, (1993) and Hassan and Raghab (1996). *Penicillium, Aspergillus* and *Geotrichum* were found to be commonly isolated from different meat products (Abdel-Rahman *et al.*,1984; Roushdy *et al.*,1996; and Hussein *et al.*,1997).

Aspergillus (12 species) was the most prevalent genus contaminating 85% and 100% of the samples of luncheon meat and poultry and comprising 38.5% and 56.9% of the total fungi respectively. Among its species *A.niger, A.parasiticus* and *A.vlavus* were the most common. Other members of Aspergillus could be isolated but in lower frequency such as *A.carbonarius, A.candidus, A.Terreus* and *A.japanicus* (Table 1). These findings were nearly similar to the results that recorded by several researchers. About 70%-84% of total luncheon samples examined were found contaminated by *Aspergillus flavus* in Assiut (Zohri, 1990; Aziz and Youssef, 1991; Farghaly, 1993; Zaki *et al.*,1995 and Hassan and Ragheb, 1996). Nahed (1999) recorded that 81.09% of luncheon were contaminated with Aspergillus, where 37.16 out of them was *A.niger.*

Penicillum occupied the second prevalent genus. It was encountered in 80 and 70% of the sample matching 23.9% and 18.8% of the total fungi on two types of luncheon respectively (Table 1). These results are in harmony with that recorded by several researchers. Abdel-Rahman and El-Bassiony (1984) detected *Penicillium* spp. especially *P.verrucosum var cyclopium* in luncheon in 94.5%, while Reiss (1986) detected *Penicillum* sp. in meat products, Zohri (1990) detected *Penicillum* of 20 species and 2 species of mucor from luncheon samples. Hassanien (1996) and Roushdy *et al.* (1996) found that Aspergillus, Penicillum were the most common species in luncheon. Ismail and Zaki (1999) found *P.variabile* and *P.janczewskii* in high percentage in luncheon meat. Alternaria (2 species) was the third frequent genus

contaminating 35% and 50 % of total samples constituting 5% and 7% of total fungi on luncheon meat and poultry, respectively. Mucor (2 species) was also common and recovered from 50% and 30% of the samples constituting 10.3% and 7.5% of total fungi on the two types of luncheon. *M.circinelloides* was the most common while *M.rascemosus* was less frequent. The remaining fungi were less frequently encountered (Table 1).

Most of these fungi had been isolated previously, but with different frequencies from meat products (Hitokoto *et al.*, 1972; Abdel-Rahman *et al.*,1984; Zdenka and Pepeljnjak, 1986; El-Khateib and Abdel-Rahman 1989; El-Maraghy and Zohri, 1995, 1996; Ismail and Zaki, 1999.

Capabilities of fungi for enzyme production:

A total of 54 isolates, belonging to 26 species were tested for their ability to produce lipase and protease enzymes. Of these isolates 44 and 39 only were able to produce lipase and protease enzymes, respectively (Table 3).

Lipase production:

Of the 44 positive isolates, 15 showed high activity, while 23 revealed moderate lipase activity. The other 6 isolates had weak activity. These isolates belonged to five species, *Alternaria alternata* (1 isolate), *A. flavus* (1), *A. niger* (1), *A. parasiticusi* (2) and *Paecilomyces variotii.*

Protease production:

The protease enzyme was detected by 39 isolates of which 10 were highly producers (*Alternaria alternata,* (1 isolate); *A.chlamydospora,* (1); *Aspergillus alutaceus,*(2); *A.aureolortus,* (1); *Mucor circinelloides,* (2); *Paecilomyces variotii,* (1); *Rhizoctonia solani,* 1 and *Rhizopus* stolonifer, (1). On the other hand, 10 isolates of four species, *Alternaria alternata* (2), *Aspergillus flavus* (4), *A.parasiticus* (3) and *Geotrichum candidum* (1), were moderate producer activity. The remaining 19 isolates showed low activity.

Many researches concerned with the ability of fungi to produce lipase and protease enzymes. Abdel-Rahman and Saad (1989) ; Banwart (1989) found that fungi isolated from meat and meat products e.g. *Penicillium, Mucor, Cladosporium, Fusarium, Aspergillus, Geotrichum, Alternaria* and *Rhizopus* had lipolytic and proteolytic activity.

Megella *et al.* (1990) found that some isolates of *Penicillum chrysogenum, Aspergillus flavus* and other species exhibited high proteolytic activity.

Abdel Sater and Ismail (1993) showed that 72.5% of 69 isolates had the ability to produce caseinase enzyme. They observed that the isolates of *Aspergillus alutaceus, Chastomium globosum, Cladosporium sphaerospermum, Emericella nidulins var lata* and *Penicillum chrysogenum* produced caseinase enzyme in strong degree. Lipase and protease enzymes were produced by several isolates of fungi in variable degrees (Trigueros *et al.*,1995; Vanderzant and Moore, 1995; Yadar *et al.*, 1998; Barakat and Abdel-Sater, 1999; Abbas *et al.*, 2002; Cabaleiro *et al.*, 2002; Papaglanni and Moo-Young, 2002; Aalbaek *et al.*,2002 and Germano *et al.*, 2003).

In conclusions, a large number of moulds species including mycotoxic fungi were isolated from both luncheon meat and poultry, such fungal contamination make the products unpalatable and unsafe for consumption (Munimbazi and Bullerman, 1996).

The results indicated improper plant sanitation and neglected hygienic measures during production packing or storage. Also it was observed that most isolates tested had variable levels of proteolytic and lipolytic activities.

To avoid such contamination, educational programs and training courses should be recommended to the meat handlers and workers. The meat additives should be conditioned and checked periodically for the presence of moulds. Sanitary rules should be adopted and periodical cleaning and disinfecting of transport vehicles and meat cold-stores.

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Table 1: Fungi isolated from 40 luncheon samples meat and poultry (20 samples each).

Genera & species	Luncheon meat			Luncheon poultry		
	TC	TC %	NCI &OR	TC	TC%	NIC&OR
Alternaria	19	5.1	7 M	15	7	10 M
A. Alternata (Fries) Keissier	18	4.8	7 M	15	7	10 M
A.chlamydospora Muchacca	1	0.3	1 L	-	-	-
Aspergillus	142	38.5	17 H	122	56.9	20 H
A.alutaceus Berkely & Curtis	2	0.54	2 L	1	0.5	1 L
A.aureolatus Munt. (Vet & Bata)	1	0.3	1 L	6	2.8	3 L
A.candidus	4	1.1	4 L	-	-	-
A.carbonarius Bainier & Thom	16	4.3	5 L	-	-	-
A.flavus Link	41	11	12 H	17	17.8	11 H
A.funigatus Fresenius	1	0.3	1 L	-	-	-
A.japanicus Saito	-	-	-	6	2.8	3 L
A.melleus Yukawa	2	0.54	1 L	-	-	-
A.niger Van Tieghem	53	14.5	14 H	66	30.8	17 H
A.parasiticus Speare	20	5.4	9 M	20	9.4	9 M
A.tamarii Kita	2	0.54	1 L	-	-	-
A.terreus Thom	-	-	-	6	2.8	4 L
Cladosporium cladesporiodes (Fresenius) de Vries	13	3.5	4 L	-	-	-
Cunninghamella elegans Lendner	8	2.2	2 L	3	1.4	2 L
Drchslera spicifera Nelson	-	-	-	4	1.9	1 L
Emericella nidulans (Eidam) Vuillemin	2	0.54	1 L	4	1.9	1 L
Epicoccun nigrum Link	2	0.54	2 L	-	-	-
Fusarium solani (Martius) Saccardo	3	0.8	1 L	4	1.9	3 L
Geotrichum candidum Link	27	7.3	4 L	-	-	-
Mucor	38	13.25	11 H	16	7.5	6 M
Mucor circinelloid Van Tieghem	31	10.25	10 M	16	7.5	6 M
M.racemosus Fresenius	7	3	1 L	-	-	-

Table 1:

Genera & species	Luncheon meat			Luncheon poultry		
	TC	TC %	NCT&OR	TC	TC%	NIT&OR
Penicillum	88	23.9	16 H	40	18.8	14 H
P.brevicompactum **Dierckx**	2	0.54	1 L	24	11.2	8 M
P.chrysogenum **Thom**	50	13.4	11 H	-	-	-
P.citrinum **Thom**	1	0.3	1 L	-	-	-
P.corylophilum **Dierckx**	20	5.4	4 L	7	3.3	4 L
P.duclauxii **Delacroix**	-	-	-	4	1.9	3 L
P.islandicum **Sopp**	3	0.8	1 L	-	-	-
P.itali **Wehmer**	1	0.54	1 L	-	-	-
P.variable **Sopp**	-	-	-	4	1.9	2 L
Pen. Sp.	11	2.96	1 L	1	0.5	1 L
Rhizoctonia solani **Kühn**	11	2.96	4 L	5	2.3	4 L
Rhizopus stolonifer **(Ehrenberg) Vuillemin**	19	5.1	6 M	1	0.5	1 L
Scopulariopsis breviculis **Saccardo (Bainier)**	1	0.3	1 L	-	-	-
Total count	373			214		
No. of genera 14	13			10		
No of species 35	30			20		

TC = Total count calculated per 240 segments

TC% = Total count percentage calculated per total count of fungi

NCI = Number of cases of isolation out of 20 samples examined

OR = Occurrence Remoras

L = Low 1-5 cases

M = Moderate 6-10 cases

H = High 11-20 cases

Table (2): Minimum (Min), maximum (Max), mean and standard deviation (SD) of the common fungi from both luncheon meat and poultry.

Species	Luncheon meat				Luncheon poultry			
	Min.	Max	Mean	SD	Min	Max	Mean	SD
Alternaria alternata	00.	6.00	0.90	1.8561	00.	3.00	0.75	0.966
Aspergillus	00.	20.00	7.10	5.9463	2.00	14.00	6.10	3.3701
A. flavus	00.	12.00	2.05	3.1368	00.	4.00	0.85	1.0894
A.niger	00.	6.00	1.70	1.8382	00.	9.00	3.30	2.5152
A.parasiticus	00.	6.00	1.00	1.5218	00.	5.00	1.00	1.5560
M.circinelloides	00.	9.00	1.85	2.6213	00.	6.00	0.80	1.5424
Penicillium	00.	19.00	4.40	4.8384	00.	6.00	2.00	2.0520
P.corylophilum	00.	9.00	1.00	2.4279	00.	3.00	0.35	0.8127
Total	4.00	41.00	20.95	9.3159	4.00	34.00	15.15	7.7478

Table 3: Capabilities of producing lipase & or protease by common fungal species isolated from luncheon meat and poultry.

Organisms	NIT	Lipase				Protease			
		P	W	M	H	P	W	M	H
Alternaria alternata	4	4	1	1	2	4	1	2	1
A.chlamydospora	1	-	-	-	-	-	-	-	1
Aspergillus alutaceus	2	2	-	-	2	2	-	-	2
A.aureolatas	1	1	-	1	-	1	-	-	1
A.carbonorius	1	1	-	1	-	-	-	-	-
A.flavus	6	5	1	4	-	6	2	4	-
A.fumigatus	1	1	-	-	1	-	-	-	-
A.japanicus	3	1	-	1	-	2	2	-	-
A.niger	3	3	1	2	-	3	3	-	-
A.parasiticus	5	4	2	2	-	5	2	3	-
A.terreus	2	2	-	2	-	1	1	-	-
Cunnigh.elegans	1	1	-	-	1	-	-	-	-
Emericella nidul	2	1	-	-	1	1	1	-	-
Drechster aspicif	1	1	-	1	-	-	-	-	-
Geotrichum cand	2	1	-	1	-	2	1	1	-
Mucor circinelloid	3	3	-	2	1	3	1	-	2
Pen. Variotii	1	1	1	-	-	1	-	-	1
Pen.brevicomp	3	3	-	1	2	-	-	-	-
P.chrysogen	2	2	-	1	1	1	1	-	-
P.citrinum	1	1	-	-	1	-	-	-	-
P.coryloph	2	2	-	1	1	2	2	-	-
P.duclwxii	1	-	-	-	-	-	-	-	-
P.island	1	-	-	-	-	-	-	-	-
P.variabile	1	1	-	-	1	-	-	-	-
Rhizoct.soloni	2	1	-	1	-	2	1	-	-
Rhizop. Stolon	2	2	-	1	1	2	1	-	1
Total isolates	**54**	**44**	**6**	**23**	**15**	**39**	**19**	**10**	**10**
% Total isolates		**81.5**	**11.1**	**42.6**	**27.8**	**72.2**	**35.1**	**18.5**	**18.5**

NIT = Number of the isolates tested

P = positive isolates

W = weak producer

M = Moderate producer

H = High producer