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OCCURRENCE OF LIPOLYTIC AND PROTEOLYTIC FUNGI IN LOCALLY SMOKED FISH

(With 5 Tables)

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تواجد الفطريات المحللة للدهون والبروتين فى الأسماك المدخنة المحلية0

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تم استبيان مدى تلوث الأسماك المدخنة المحلية المطروحة بالأسواق بالفطريات كذلك الكشف عن قدرة الفطريات المعزولة على افرازانزيمات تحلل الدهون والبروتين والتى تؤدى الى فساد الأسماك0 تم جمع عدد 100 عينة من اسماك الرنجة المدخنة المغلفة والغير مغلقة من السوبر ماركت المختلفة فى مدينة بورسعيد 0 واسفرت نتائج الفحص ان نسبة كبيرة من العينات ايجابية العزل للفطريات وخاصة لوجود الخمائر 0 وقد تبين من الفحص ان معظم العترات المعزولة تنتمى الى جنس الاسبرجلس والبنسيليوم والكانديدا 0 وقد اظهرت معظم العترات المختبرة القدرة على تحلل الدهون والبروتين بنسب متفاوتة قد تصل فى بعض العترات من 95 -100% كما هو الحال فى اجناس البنسليوم والروديترا والميكوروالديباريوميسيس، كذلك اظهرت بقية العترات المعزولة مثل اجناس الكانديدا والاسبرجلس وبعض العترات الأخرى نتيجة ايجابية لتحلل البروتينات والدهون بنسب مختلفة مما يدل على الدور الذى تلعبه تلك الميكروبات فى فساد الأسماك المدخنة0 هذا وقد تم مناقشة العوامل المختلفة التى تؤدى إلى زيادة نمو تلك الميكروبات وأثرها على الصحة العامة**0**

SUMMARY

A total of 100 of locally smoked fish samples, packed in polythene bags or unpacked (50 of each) were subjected to mycological examination. The proteolytic and lipolytic potentials of fungi isolates associated with examined smoked fish were investigated. The incidence of yeast was higher than mould in either packed or unpacked smoked fish samples. *Aspergillus* and *Pencillium* spp. were the dominant mould groups, irrespective of the packed or unpacked samples. Other moulds isolated were *Eurotium, Fusarium, Mucor* and *Cladosporium* species. The predominant yeast species was *Candida* followed by *Debaryomyces* spp., *Rhodotroula* spp. and *Saccharomyces* spp. The protease and lipase producing potential of the isolates varied among the genera and between

isolates of the same species. Among the isolates, strains of *P. expansum, P. viridicatum, Mucor, Rhodotroula and Debaromyces* spp. *demonstrated* the most noticeable proteolytic activity with a percentage reach up 100%, followed by strains of *A. niger* and *A. flavus.* The majority of tested mould and yeast isolates were showed lipolytic activities. The existence and growth of these fungi on smoked fish is a pointer to the potential health risk associated with the consumption of smoked fish. Their ability to elaborate protease and lipase may also an indication of their active role in the spoilage of smoked fish.

Key words: *Fungi, smoked fish, mould, yeast*

INTRODUCTION

Smoking is one of the oldest methods of preserving fish, or any other meats for that matter. Long before there were no refrigerators and freezers, fishing ancestors learned to use a combination of salt and smoke to keep fish from spoiling. Today, smoking is no longer necessary, but it remains popular for the flavor it gives to fish. Smoking methods vary, but all are based on a few common principles. First, the fish is treated with salt, either in the form of strong brine or a surface coating of dry salt. Most fish are given a second cure after the initial salting to add additional flavors. After curing, the fish is rinsed to remove the salt and other curing ingredients from the surface, then allowed to dry in cool flowing air until a shiny, slightly tacky skin (pellicle) forms on the surface. The actual smoking takes place inside a chamber filled with smoke from smoldering hardwood. At this point the process diverges; fish are either "hot-smoked" or "cold-smoked," depending on the temperature of the smoking chamber. The choice determines the texture, flavor, and potential uses of the fish (AFDO, 1991).

From the processing to the market, smoked fishes are often contaminated with microorganisms including mould and yeast (Okafor and Nzeako, 1985 and Wu and Salunkhe, 1978). Fungal contamination is considered the main spoilage agent of smoked fish, impart musty off-flavour, sliminess, lipolysis and unpalatable taste that render the products of inferior quality, unmarketable or even unfit for human consumption (Stoskopf *et al*., 1993). The spoilage of smoked fish during storage is considered an important and dangerous problem facing smoked fish producers. Smoked fish are more susceptible to hydrolysis and oxidation due to lipolytic moulds and yeasts which are capable of

causing these deteriorations (Ward and Baaji, 1988). The degree of spoilage of yeasts and moulds isolated from protein – rich foods such as sea foods, can be estimated according to the proteolytic activity of the isolated fungi (Kobatake *et al*., 1992).

In view of this concern, the present work was carried out to investigate the degree of fungal contamination in smoked fish and to throw the light on the proteolytic and lipolytic potentials of fungi associated with smoked fish.

MATERIALS and METHODS

1. Samples Collection:

A total of 100 locally smoked Herring fish samples, 50 each from, packed in polythene bags and unpacked, were collected randomly from retail markets of different sanitation levels in Port Said City. The collected samples were dispatched directly to the laboratory in an ice box where they were prepared and examined.

2. Mycological examination:

2.1. Preparation of homogenate:

Fish sampling and preparation of sample homogenate were carried out according to the technique recommended by Andrews and Hammack (1998).

2.2. Enumeration of total moulds and yeasts:

The mould and yeast contamination levels of the examined samples were determined by using dilution plate technique. Malt extract agar (Difco) and potato dextrose agar (Difco) plates with added 100 ppm Chloramphenicol were inoculated with appropriate dilutions (Tournas *et al*., 2001). The plates were incubated at 28^0C for one week at which resulting fungal colonies were counted separately by naked eye and the mould and yeast counts per gram were calculated and recorded.

On the other hand, colonies with different morphological characters were inoculated on appropriate media for purification and isolation.

2.3. Identification of the isolates:

Identification of the mould genera and species based on macroscopical and microscopical features was carried out according to the recommended methods outlined by Pitt and Hocking (1985) for penicillia, Raper and Fennell (1965) for aspergilli. Other Deuteronomycetes, Ascomycetes and Zygomycetes were identified as reported by Samson *et al.* (1981) and Pitt and Hocking (1985).

Besides, the isolated yeasts were identified according to the technique recommended by Campbell *et al*. (1980).

3-Determination of proteolytic activity of the fungal isolates:

Proteolytic activity of fungi was determined as described by ICMSF (1978). Each fungal isolate was inoculated on the surface of skim milk agar in which skim milk was added just before pouring the medium into the Petri- plates. Plates were incubated for 7 days at 28^0C; afterwards, the clear zones of hydrolysis (degradation of milk protein around the colony) were measured and recorded as zone of hydrolysis.

4-Determination of lipolytic activity of isolates (Koburger and Jaeger, 1987)

Each mould or yeast isolate was inoculated on the surface of tributyrin agar plates. The inoculated plates were incubated at 25^0C for one week. Yeast or mould colonies showing lipolytic zone were recoded.

RESULTS

Table 1: Statistical values of total mould count in the examined smoked fish samples (n=50 each).

Smoked fish	Mould count (cfu/g)				
	Positive		Min	Max	Mean ±SE
	No	%			
Packed	35	70	<10	$2.1X10^3$	$5X10^2 \pm 8X10$
Unpacked	42	84	$1X10^2$	$1X10^4$	$2.5X10^3 \pm 3X10$

Table 2: Incidence of mould species isolated from the examined smoked fish samples.

Mould species	N0. of isolates		Total isolates (%)
	Unpacked smoked fish	Packed smoked fish	
Aspergillus spp	39	52	91(67.4)
A. niger	20	18	38 (28.1)
A. flavus	5	13	18 (13.3)
A. fumigatus	7	10	17 (12.6)
A. ochraceus	2	7	9 (6.7)
A. candidas	5	4	9 (6.7)
Pencillium spp	19	9	28(20.7)
P. expansum	12	9	21(15.6)
P. viridicatum	7	0	7 (5.2)
Mucor spp.	0	3	3 (2.2)
Fusarium spp.	0	4	4 (3)
Eurotium repens	2	4	6 (4.4)
Cladosporium spp.	1	2	3 (2.2)
Total	61	74	135 (100)

NB: Percentage was calculated according to the total number of mould isolates (135).

Table 3: Statistical values of total yeast count of the examined smoked fish samples (n=50 each).

Smoked fish	Yeast count(cfu/g)				
	Positive		Min	Max	Mean ±SE
	No	%			
Packed	50	100	3X10	$3.5X10^4$	$5.2X10^3 \pm 3X10$
Unpacked	46	92	<10	$1.2X10^4$	$1.2X10^3 \pm 2X10^2$

Table 4: Incidence of yeast species isolated from the examined smoked fish samples.

Yeast species	N0. of isolates		Total isolates (%)
	Unpacked smoked fish	Packed smoked fish	
Candida spp.	34	48	82(61.6)
C. albicanis	20	28	48 (36.1)
C. lipolytica	7	10	17 (12.8)
C. tropicalis	7	10	17 (12.8)
Saccharomyces spp.	5	7	12 (9)
Rhodotroula spp.	8	9	17 (12.8)
Debaryomyces spp.	12	10	22 (16.5)
Total isolates	59	74	133 (100)

NB: Percentage was calculated according to the total number of yeast isolates (133).

Table 5: Proteolytic and lipolytic activities of fungi isolated from the examined smoked fish samples.

Isolated fungi	N0. of isolates	Proteolytic		Lipolytic	
		No.	%	No.	%
A. niger	38	33	86.8	20	52.6
A. flavus	18	15	83.3	16	88.9
A. fumigatus	17	12	70.6	12	70.6
A. ochraceus	9	4	44.4	6	66.7
A. candidas	9	3	33.3	5	55.6
P. expansum	21	21	100	2o	95.2
P. viridicatum	7	7	100	7	100
Mucor spp.	3	3	100	3	100
Fusarium spp.	4	1	25	4	100
Eurotium repens	6	2	33.3	6	100
Cladosporium spp.	3	1	33.3	2	66.7
C. albicanis	20	2	10	12	60
C. lipolytica	7	1	14.3	7	100
C. tropicalis	7	2	28.6	5	71.4
Saccharomyces spp.	5	2	40	4	80
Rhodotroula spp.	8	8	100	8	100
Debaryomyces spp.	12	12	100	12	100

DISCUSSION

The present data in Table (1) revealed the incidence of mould in the examined samples. Moulds were recovered from packed and unpacked smoked fish at a rate of 70 % and 84 %, respectively. The mean mould counts were $5x10^2 \pm 8x10$ and $2.5\ x10^3 \pm 3x10$ cfu/g for packed and unpacked examined smoked fish samples, respectively. The obtained findings are coincided with those recorded by Edris (1996) and Lashin (2004). The low moisture content of smoked fish favors the growth of mould (Connell, 1990). Mould contamination may be attributed to inadequate processing, recontamination or incorrect storage of herring after processing. In this respect Graikoski (1973) reported that smoked fish can be contaminated with mould mainly from contaminated chamber and wood smoke as well as dust used in fish smoking. The relatively higher mould count of unpacked smoked fish samples may be attributed to continuous contamination of the fish through frequent handling and to the general poor sanitary condition of many markets.

The examined smoked fish samples harbored more mould genera and species (Table 2). *Aspergillus* and *Pencillium* spp. were the dominant groups irrespective of the packed or unpacked samples. Out of the 135 moulds isolated from the packed and unpacked smoked fish samples, *A .niger* was the dominant with an incidence of 28.1% of the total mould isolates. *Aspergillus flavus and Penicillium expansum* were also frequently isolated. Other moulds isolated were *Cladoporium, Eurotium, Fusarium and Mucor* species. The obtained results of the isolated moulds are in agreement with those obtained by Munimbazi and Bullerman (1996); Pangi *et al*. (1990) and Yousef (1998).

It is worthwhile from the recorded results that most of the isolated moulds are toxigenic types and have the ability to produce mycotoxins whenever the conditions are right and become of public health hazard. *A. flavus* is known to produce aflatoxins, while *P. expansum* and *P. viridicatum* produce penicillic acid and ochratoxin respectively when cultured on organic substrates (Joffe, 1965). Of the remaining genera, *Eurotium repens* have been associated with the production of sterigmatocystin. Fusaria species produces trichothecenes and zearalenone (Davis and Diener, 1978 and Marasas *et al*., 1979) while *Cladosporium* species are known to produce epicladosporic acid and fagicladosporic acid (Joffe 1965).

Yeasts were recovered from packed and unpacked smoked fish at a rate of 100 % and 92 %, respectively (Table 3). The mean yeast count of the examined samples were $5.2x10^3 \pm 3x10$ and $1.2\ x10^3 \pm 2x10^2$ cfu/g for packed and unpacked smoked fish samples, respectively. The

present results coincide with that reported by El- Sayed (1995). In this concern, Nickelsen and Finne (1992) reported that yeast form a significant proportion of the spoilage flora in smoked products, with high heat in put where yeasts are the more stable organisms that will be predominant. It is obviously that the high incidence of yeast contamination was noticed in packed smoked fish, packing may increase contamination by yeasts. Similar findings were reported by Kemp *et al.* (1986).

Dealing with the isolated yeast species, the achieved results in Table (4) showed that the predominant yeast species isolated from the examined samples was *Candida* spp. Out of 133 yeast isolates, 22(16.5%) were belonging to *Debaryomyces* spp., 17*(*12.8%) were belonging to *Rhodotroula* spp. and 12(9%) were belonging to *Saccharomyces* spp., while *Candida* spp. comprised the remaining 61.7%. Such isolated yeasts could be recovered by other investigators from smoked fish (Ibrahim, 2000 and Lashin, 2004). The isolated yeast species were mentioned as spoilage organisms of smoked fish with undesirable changes during prolonged storage (Coni *et al*., 1994). From the public health point of view the most predominant yeast species was *Candida albicanis* (36.1%) which constitute a public health hazard as involved in several allergic conditions, pulmonary infection, vulvovaginitis, endocarditis, meningitis and occasionally fatal systemic disease (Kwon- Chung and Benett, 1992).

Proteolytic activity of isolates

The results recorded in Table (5) pinpoint that most of the isolated fungi have a proteolytic potential. Their proteolytic activity however varies among the isolates. Among the isolates, strains of *P. expansum* and *P. viridicatum* and *Mucor* spp. demonstrated the most noticeable proteolytic activity (100%), followed by strains of *A. niger* (86.8%) and *A. flavus* (83.3%). On the other hand, *A. fumigatus, A. ochraceus and A. candidas* showed relatively lower percentage of proteolytic activities. *Cladosporium* spp, *Eurotium repens* and *Fusarium* spp. exhibited low proteolytic activities, and were not frequently isolated. Among the isolated yeasts, *Rhodotroula* spp. and *Debaromyces* spp. showed the most noticeable proteolytic activity (100%). *Candida spp*. and *Saccharomyces* spp. showed low percentage of proteolytic activities ranged from 10- 40%. The obtained results agreed to certain extent with those reported by other investigators (Yousef, 1998; Sayid, 1999; Lashin, 2004).

Proteinases are a well known group of proteolytic enzymes that play an important role in food processing industry, they are considered among the most important enzymes in the breakdown of fish materials when fungi attack their surface (Ward, 1985). The ability of the isolates to elaborate protease is an indication of their active role in the spoilage of smoked fish. The level of proteolytic microorganisms may be of value to predict refrigerated storage life and to assess processing methods (Lee and Kraft, 1992).

Lipolytic activity of isolates

The data recorded in Table (5) declared that the incidence of lipolytic activity was high in most of the examined isolates. Among the mould isolates, strains of *P. expansum*, *P. viridicatum*, *Mucor* spp., *Fusarium* spp., *Eurotium repen*s were showed lipolytic activities with a percentage reach up to 95-100%. *Aspergillus* spp. and *Cladosporium* spp. showed lipolytic activity with an incidence ranging from 52.6- 88.9%. The tested yeast isolates showed lipolytic activity with an incidence ranged from 60-100 %. The obtained results are in accordance with that recorded by other investigators (Banwart, 1980, Godtfred, 1990, Sallam *et al*., 1991 and Vanot *et al*., 2001).

Fungi are known to induce certain objectionable changes in the fat content of fish rendering them unmarketable or even unfit for consumption (Smith and Hass, 1992). The rancidity of smoked fish when stored for long time at a relatively high temperature is mainly due to lipase enzyme produced by lipolytic moulds (Watanabe and Dzekedzeke, 1971). Fungal contamination is considered the main spoilage agent of smoked fish which lead to impart musty off flavors, sliminess, lipolysis and unpalatable taste (Welthagen and Viljoen, 1999).

Smoked fish is now a product of general consumption. The spoilage of smoked fish during storage is considered an important and dangerous problem facing smoked fish producers. The risk coming from fungal growth in relation to smoked fish that all parameters required for mould growth are available specially where the product badly packed, not protected from environment and stored at ordinary room temperature. The existence of moulds and yeasts that can produce proteases and lipase in smoked fish strengthened the possibility of smoked fish to undergo spoilage, unmarketable or even unfit for human consumption that may constitute a public health hazard and economic losses. Fungal contamination of smoked fish should be viewed with serious concern because of the ability of the moulds to produce mycotoxins, some of which are very dangerous and lethal to humans

even in small doses. Proper storage of smoked fish is also necessary because poor storage methods and unhygienic handling of the items are known to predispose them to fungal contamination.

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