Studies On The Presence Of Fungi In Some Salted And Smoked Fish With Special Reference To Proteolytic And Lipolytic Strains

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ABSTRACT

Fifty random samples (10 of each) of salted fish [Moloha, Fessekh and Sardin] and smoked fish [smoked herring and smoked salmon] were collected from different markets of Giza and Monofia governorates and examined to Evaluate the moulds and yeasts contamination. The mean value of mould count of examined fish samples Moloha, Fessekh, Sardin, smoked herring and smoked salmon were 8.4 X 10^3, 2.7 X 10^3, 6 X 10^2, 1 X 10^4 and 5 X 10^3 respectively while the mean value of yeast count were 5.5 X 10^3, 8.7 X 10^3, 7.7 X 10^4, 2.9 X 10^4 and 3.2 X 10^4 respectively for all kinds.

The identification of mould isolates revealed the presence of *A. flavus*, *A. niger*, *A. parasiticus* and *Penicillium vercosum* in percentage of 28.5% for *A. flavus*, *A. niger* and *Penicillium vercosum* while *A. parasiticus* presenting 14.5% of examined samples. The yeast isolates present as *C. tropicals*, *C. pseudotropicals*, *C. albicans*, *C. lipolytica*, *C. zeylanoides* and *Rhodotorula glutinis* in percentage of 25%, 28.6%, 10.7%, 21.4%, 10.7% and 3.6%, respectively.

The proteolytic and lipolytic activity were examined for both moulds and yeasts isolates. The public health importance and hygienic significance of isolated organisms were discussed as well as the control measures to improve the quality of such products.

INTRODUCTION

Fish is one of the most perishable commodities world wide and could considered as replace of meat and poultry in human diet for its high nutritive due to its high biological value of its protein which contains essential amino acids, mineral, vitamins trace elements and polysaturated fatty acids.

Salting and smoking of fish are used long before other preservation techniques. They are less expensive than other methods and suitable for developing countries.

Fish salting is a traditional technique used since ancient Egypt for fish preservation and flavoring. The preservation occurs by rendering the fish unsuitable for microbial proliferation by reducing the water activity through fish dehydration *(1)*. During most of the salting operation fish are maintained in a damp condition which provide ideal growing condition for moulds and yeasts.

Smoking is also very old preservation process and still widely used to impart flavor and/or color to some food products rather than preservation. The most common type of fish used for smoking are herring and salmon as they have high protein and fat content. Smoking is a complete of more than 200 compounds including antioxidative and bactericidal ingredients *(2)*.

Fungal contamination is considered the main spoilage agent of smoked and salted fish imparts off tastes that render the product of inferior quality unmarketable or even unfit for human consumption and may constitute a public health hazard due to toxic metabolites which may cause mutagenic, carcinogenic and teratogenic effect *(3)*.

The risk coming from fungal growth in relation to salted and smoked fish is that all parameters required for growth and toxic production are available specially where the products being badly packed, non protected from environment and stored at ordinary room temperature. The quality of fish products depends on a large degree on condition of the raw material *(3)*. Therefore this study was done to throw light on the degree of contamination of such food products with moulds and yeasts.

MATERIAL AND METHODS

Collection of samples

Fifty (50) random samples of salted fish (Moloha, Fessekh and Sardin) and smoked fish (Herring- smoked salmon) 10 samples of each were collected from Giza and Mnofia governorates.

Preparation of the samples

Fish samples were prepared according to the previous recommended technique *(4)*. A known area (10 cm^2) of skin by using a template was removed as follows: after making incisions the skin was removed from the muscle with sterile forceps and scalpel.

To 10 g of fish 90 ml of sterile peptone water were added and thoroughly mixed in a sterile warning blender, the mixture represented the dilution of 10^{-1} from which fold serial dilutions were accomplished.

Total mould and yeast counts

The recommended method for mould and yeast counts *(5)* was adopted. Sabaurouds dextrose agar medium *(6)* supplemented with chloramphenicol 0.05 mg/ ml was used and incubated at 25°C for 5 days.

Identification of mould isolates

The identification of the mould genera and species were carried out by careful observation and measurements of macroscopic and microscopic characteristics of the mould colonies *(7) and (8)*.

Identification of yeasts

Yeast identification by microscopy on corn meals as well as fermentation and assimilation were performed according to the recommended technique *(9)*.

Preteolytic activity of isolates

Proteolytic activity was determined using a casein substrates as previously described *(10)*. The plates were incubated at 28°C for 7 days.

Lipolytic activity of isolates

Lipolytic activity was determined using tributyin agar medium according to the recommended technique *(11)*.

RESULTS AND DISCUSSION

The results obtained in Table 1 revealed that the mean value of total mould count/ cm^2 were 8.4 X 10^4, 2.7 X 10^3, 6 X 10^2 of examined salted fish [Moloha, Fessekh and Sadin] respectively.

The same table showed the mean value of total yeast count/ cm^2 presented as 5.5 X 10^3, 8.7 X 10^5, 7.7 X 10^4 for the salted fishes respectively. The lower results were recorded *(12- 14)*.

The results for smoked fish samples [smoked herring and smoked salmon] were for mean value of total mould count 1 X 10^4 and 5 X 10^3 while for yeast count were 2.9 X 10^4 and 3.2 X 10^4. This result higher than those obtained *(3, 13-17)*.

Fungal contamination of fish could be attributed to improper sanitation during catching, handling, manufacturing storage and marketing of fish *(18)*.

The yeast form a significant proportion among isolates and it was found that raw fish contaminated with yeasts species produce heavy yeast contaminated products on other hand the long term deep frozen storage shows no significant changes in the level of yeast contamination. So some countries considered the mould and yeast count more or less as an indicator for hygienic measures adopted during the handling of foods.

Table 2 revealed that the incidence and identification of isolated moulds from examined fish samples and were *Aspergillus flavus*, *Aspergillus niger* and *Penicillium vercousm* in percentage of 28.5% while *Aspergillus paraciticus* was 14.5%. This obtained results nearly agreed with those reported by *(3, 13, 15, 19- 21)*.

Table 1. Statistical analysis for moulds and yeasts count / cm^2 of the examined fish samples.

Fish sample	Mould count						Yeast count				
	No. of exam. samples	No. of +ve samples	%	Min.	Max.	Mean	No. of +ve samples	%	Min.	Max.	Mean
Moloha	10	3	30	4×10^2	2×10^4	8.4×10^3	2	20	1×10^3	1×10^4	5.5×10^3
Fessekh	10	3	30	1×10^2	6×10^3	2.7×10^3	4	40	1×10^2	2.8×10^4	8.7×10^3
Sardine	10	2	20	1×10^2	1.1×10^3	6×10^2	6	60	9×10^3	2.9×10^5	7.7×10^4
Smoked herring (Renga)	10	3	30	1×10^2	3×10^4	1×10^4	6	60	1×10^2	9×10^4	2.9×10^4
Smoked salmon	10	3	30	1×10^2	1×10^4	5×10^3	10	100	3×10^2	2.7×10^5	3.2×10^4

Table 2. Incidence and identification of mould isolates from the examined fish samples (No. of isolates = 14).

Isolates	+ve isolate	%	Salted fish			Smoked fish	
			Moloha	Fessekh	Sardine	Smoked herring	Smoked salmon
A. flavus	4	28.5	2	1	-	-	1
A. niger	4	28.5	-	1	1	-	2
A. parasiticus	2	14.5	1	-	-	1	-
Penicillian vercosum	4	28.5	-	1	1	2	-
Total	14	100	3	3	2	3	3

From the obtained results some mould were recorded as toxigenic types and have the ability to produce mycotoxins whenever the condition are favorable and become a public health hazard e.g. *Aspergillus flavus* and *Aspergillus parasiticus* by virtue of their ability to produce aflatoxins which have considered the most potential liver carcinogenic agent known till now in human being ***(22)***.

In addition some species of genus Penicillium included pulmonary infection, external otomyciesis and endocarditis ***(23)***.

Table 3 revealed incidence of yeast isolated from examined fish samples as following *C. tropiculs, C. pseudotropicals, C. albicans, C. lipolytico, C. zenylonoides* and *Rhodotorola glutinis* in 25%, 28.6%, 10.7%, 21.4%, 10.7% and 3.6% respectively. These results agreed to the results previously recorded ***(14, 24 , 25)***.

From the public health of view some species of yeast constitute a public health hazard as *Candida albicans* which was not only responsible for active infection but also involved in several allergic conditions ***(26)***.

Table 4 showed the results of preteolytic and lipolytic activity and revealed the isolated members from the genus Aspergillus have proteolytic activity in 100% of isolates while penicillium species showed activity 50% of the isolates similar results we obtained several investigators ***(27,28)*** while lipolytic activity was recorded in all isolates (100%) results agreed with those cited by ***(29,30)***.

Table 3. Incidence and identification of yeasts isolates of the examined fish samples (No. = 28)

Isolates	+ve isolate	%	Salted fish			Smoked fish	
			Moloha	Fessekh	Sardine	Smoked herring	Smoked salmon
C. tropicals	7	25	1	1	2	1	2
C. pseudotropicals	8	28.6	-	1	2	2	3
C. albicans	3	10.7	-	-	1	-	2
C. lipolytica	6	21.4	1	2	-	3	-
C. zeylanoides	3	10.7	-	-	1	-	2
Rhodotorula glutinis	1	3.6	-	-	-	-	1
Total	28	100	2	4	6	6	10

Table 4. Protolytic and lipolytic activity of isolated mould from examined fish samples (No. of isolates = 14)

Isolates	Proteolytic activity										Lipolytic activity									
	Moloha		Fessekh		Sardine		Smoked herring		Smoked salmon		Moloha		Fessekh		Sardine		Smoked herring		Smoked salmon	
	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
A. flavus	2	100	1	100	-	-	-	-	1	100	2	100	1	100	-	-	-	-	1	100
A. niger	-	-	1	100	1	100	-	-	2	100	-	-	1	100	1	100	-	-	2	-
A. parasiticus	1	-	-	-	-	-	1	-	-	-	1	100	-	-	-	-	1	100	-	-
Penicillium vercosum	-	-	1	100	1	100	2	50	-	-	-	-	1	100	1	100	2	100	-	-

Table 5. Protolytic and lipolytic activity of isolated yeast from examined fish samples (No. of isolates = 28)

Isolates	Proteolytic activity										Lipolytic activity									
	Moloha		Fessekh		Sardine		Smoked herring		Smoked salmon		Moloha		Fessekh		Sardine		Smoked herring		Smoked salmon	
	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
C. tropicals	1	-	1	-	2	50	1	-	2	50	1	100	1	100	2	100	1	100	2	100
C. pseudotropicals	-	-	1	-	2	-	2	-	3	-	-	-	1	100	2	100	2	100	3	100
C. albicans	-	-	-	-	1	-	-	-	2	-	-	-	-	-	1	100	-	-	2	100
C. lipolytica	1	-	2	-	-	-	3	-	-	-	1	100	2	100	-	-	3	100	-	-
C. zeylanoides	-	-	-	-	1	-	-	-	2	-	-	-	-	-	1	100	-	-	2	100
Rhodotorula glutinis	-	-	-	-	-	-	-	-	1	100	-	-	-	-	-	-	-	-	1	100

The results recorded in table 5 was presented that *C. tropicals* showed proteolytic activity as 50% in both salted and smoked fish and *Rhodotorula glutinis* give 100% for the lipolytic activity all yeasts isolates give 100% of this activity which consistent with those obtained by ***Smith and Hass (31)*** .

Protein hydrolyzing microorganisms may produce a variety of odour and flavour defects particularly when contamination is high so it is necessary to give full consideration of spoilage microorganisms not only bacteria but also the mould and yeasts. The same consideration must be given to lipolytic fungi as it widely spread in nature and are heat resistant and their activity withstand in foods for long period even at low temperature *(17)*.

From the achieved results it could be concluded that the absence of visual fungal growth does not mean that the moulds and yeasts are absent. So the quality of fish and the modern preservation techniques are essential for getting a good products hence a hygenic restricted measures must be taken to avoid the growth of mould and yeast during and after processing as well as during their storage.

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الملخص العربى

دراسات عن تواجد الفطريات فى بعض الاسماك المملحة والمدخنة وبخاصة العترات المؤدية لتحلل البرتينات والدهون

منال محمد على ، أمل على شحاته ، عاطف محمد عمر
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خمسون عينة جمعت عشوائيا من اسماك مملحة (ملوحة- فسيخ- سردين) وأسماك مدخنة (رنجة – سالمون مدخن) بواقع عشرة عينات من كل نوع من أسواق محال البقالة بمحافظتى الجيزة والمنوفية. وقد أختبرت هذة العينات لمعرفة مدى تلوثها بالفطريات والخمائر وكان متوسط العد الكلى للفطريات فى الملوحة والفسيخ والسردين والرنجة والسالمون المدخن كالتالى :

٤ر٨x١٠^{٣}، ٧ر٢x١٠^{٣}، ٦x١٠^{٢} ، ١x١٠^{٤} ، ٥x١٠^{٣} ، بينما كان متوسط العد الكلى للخمائر فى كل الانواع السابقة هى: ٥ر٥x١٠^{٣}، ٧ر٨x١٠^{٣} ، ٧ر٧x١٠^{٤}، ٩ر٢x١٠^{٤}، ٢ر٣x١٠^{٤} على التوالى. وقد تم تصنيف عترات كلا من الفطريات والخمائر المعزولة من عينات الاسماك المملحة والمدخنة فكانت الفطريات من انواع الاسبرجلس والبنسليوم بينما الخمائر كانت من أنواع الكانديدا والرودوتوريللا.

وقد تم أختبار خاصية تحلل كلا من البروتينات والدهون لجميع العترات المعزولة من الفطريات والخمائر كما تمت مناقشة الاهمية الصحية وكذلك التوصيات اللازمة للنهوض بصناعة هذة المنتجات ليتم انتاجها بالطريقة الصحية والأمنه للمستهلك.