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MYCOLOGICAL STATUS OF READY-TO-EAT SALTED FISH

(With 2 Tables)

By

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الحاله الصحيه لفطريات الاسماك المملحه الجاهزه للاستهلاك

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تعتبر الأسماك المصريه المملحه (الملوحه) من الأغذيه الشعبيه فى مصر ولم تجر دراسات فطريه مفصله وكامله على هذه الأسماك من قبل فى مصر لذلك تمت هذه الدراسه . صممت هذه الدراسه على جميع ٤٥ عينه من الأسماك المملحه والمعده للاستهلاك الآدمى فى مدينة أسيوط وقد تم عزل ١٦ جنساً من الفطريات متمثله فى ٢٩ نوع بالاضافه الى صنف واحد . كانت الفطريات السائده هى الاسبرجلس (٣ ر٥٣٪ ، ٦ ر٧٥٪). البنسليوم (٤ ر٤٤٪ ، ٧ ر٦٦٪) على وسطى العزل المستخدمين وهما آجار مستخلص الشعير وآجار مستخلص الشعير المضاف اليه ٥ ر٧٪ كلوريد الصوديوم . كذلك تم عزل أجناس اخرى هى كلادوسبوريوم ، أكريمونيوم ، الالترناريا ، أيورتيوم ، رودوتوريولا ، ريزوبس بنسب مختلفه . كانت أكثر أنواع الاسبرجلس هى فلافس ، اليوتيشيس ، فيوميجاتس ، نيجر ، سيدويوس ، تيريس . بينما كانت أنواع البنسليوم هى كريزوجينوم ، وأكسمانى . سجلت أجار مستخلص الشعير المضاف اليه ٥ ر٧٪ كلوريد الصوديوم نتائج جيده فى العزل عن آجار مستخلص الشعير . كانت متوسطات المعامل الهيدروجينى ، النشاط المائى ، كلوريد الصوديوم هى ٦ ر٦ ، ٩١٠ ر ، ١٣٠٪ على التوالى . نوقشت المخاطر الصحيه لعزلات الفطريات والاجراءات اللازمه لدرء خطورتها على صحة المستهلكين .

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SUMMARY

A total of 16 mould genera representing 29 species and one species variety were isolated from forty five ready-to-eat Egyptian salted fish samples. the dominant genera were *Aspergillus* (53.3%, 75.6%) and *Penicillium* (44.4%, 68.8%) on malt extract agar and halophilic malt agar respectively. Other frequently isolated moulds were *Cladosporium, Acremonium, Alternaria, Eurotium, Rhodotorula* and *Rhizopus* in a varying percentage. The frequent identified aspergilli were *A.flavus, A. alutaceus, A. fumigatus, A. niger, A. sydowii* and *A. terreus* while for *Penicillia* were *P. chrysogenum* and *P. waksmanii.* The halophilic malt agar recommended a good fungal isolating media more than malt extract agar. The mean value of pH, aw and NaCL% were 6.6, 0.91 and 13 respectively. The significance importance of fungal isolates as well as the control measures are discussed.

Keywords: Mycology, Ready-to-eat Salted Fish.

INTRODUCTION

Fish constitute a part of protein in the diet of some people in Egypt. Salting is the most widespread and cheapest method for fish preservation, but the water activity of the salted fish is often not low enough to prevent mould spoilage.

Fungi associated with salted fish differ according to the method of salting which used, type and quality of salt and the conditions which fish stored in a country.

In many parts of the world, the mycoflora of the salted fish was studied by many investigators; *HITOKOTO* et al. *1976* in Japan, *WHEELER* et al. *1986* in Indonesia, Lu et al. *1988* in Ghana and *ATAPATTU and SAMARAJEEWA, 1990* in Sri Lanka.

In Egypt, there are few researches on the mycoflora of the salted fish *(ABDEL-RAHMAN* et al. *1988 and ABD-ALLA* et al. *1994),* therefore this investigation was planned to throw light on the fungi either count or types associated with salted fish and growth promoting parameters.

MATERIALS AND METHODS

Forty five salted fish samples of ready to-eat were collected from Assiut markets. These samples were examined for the mycological status (enumeration and identification of the associated fungi). Other factors e.g pH, aw and NaCL% were estimated.

1- Enumeration of the fungi:

10 gm of muscle was removed from each fish sample under sterile condition and blended with 90 ml sterile physiological saline in waring blender (8000 rpm) for three minutes to yeild 10^{-1} dilution, from which serial ten-fold dilutions were made up to 10^{-6}. Triplicate plates of the appropriate dilutions were used. The isolation media were malt extract agar and malt extract agar with 7.5% sodium chloride (Halophilic malt agar) *(APHA, 1984)*. The plates were incubated at 25°C for 15 days during which the grown fungi were counted, identified and calculated per gram.

2- Identification of the fungi

The grown fungi were identified based on macro-and microscopic characterstics according to *RAPER and FENNELL, 1977, PITT, 1979, DOMSCH* et al. *1980, and KOZAKIEWICZ, 1989.*

Estimation of pH: This was estimated by using Onion research digital ionalyzer apparatus, Model 701 A. Germany and according to § 35 LMBG, 1989.

Estimation of water activity (aw): This was done by using Retronic Hygroscope DT, measuring station WA-40 and according to *RÖDEL* et al. *1979.*

Estimation of sodium chloride percentage (NaCL%): This was carried out as the method described in A.O.A.C., 1985.

RESULTS

Are presented in Table 1&2.

DISCUSSION

The results in table (1) revealed that the prevalent fungal genera from salted fish were Aspergillus (53.3% and 75.6%) and *Penicillium* (44.4% and 68.9%) on malt extract agar and halophilic malt extract agar, respectively. This finding agree with that reported by *LU* et al. *1988 and ABD-ALLA* et al. *1994.* The dominant species of *Aspergillus* were *A. flavus, A.*

fumigatus, A. niger, and *A. terreus* while the dominant species of *penicillium* were *P. chrysogenum* and *P. waksmanii. ABDEL-RAHMAN* et al. *1988.* found that *Penicillium* (38.5%) and *Aspergillus* (26.3%) were the dominant genera on salted fish. The *Penicillium* spp. were half of the isolating fungi from the dried salted sardines in Tokyo *(HITOKOTO* et al. *1976).* Cladosporium (2 species) was dominant on the halophilic medium and rarely encountered on malt extract agar. Other fungi were infrequently isolated either on malt agar or/and halophilic agar media (Table 1). *PHILIPS and WALLBRIDGE (1977)* reported *Aspergillus* (six species), *Acremonium* and Rhizopus from salted fish. The most prevalent fungi on Sri Lanka dried salted fish were *Aspergillus niger, A. flavus, A. fumigatus, A. glaucus, A. restrictus, Aureobasidium sp., Basipetospora halophila, Cladosporium herbarum, Gliomastix sp., Penicillium chalybeum* and *P. expansum (ATAPATTU and SAMARAJEEWA, 1990).* Also *UDAGAWA and TSURUDA, 1975* found that *Aspergillus ochraceus, A. oryzae, A. ostianus. Penicillium cyclopium var echinulatum, P. pulterilli and Syncephalastrum racemosum* in finaly dry salted fish.

The average pH was 6.6 which is relatively higher than recorded by *ABDEL-RAHMAN* et al. *1988,* while the average NaCL% was 13 which is low than reported by *ABDEL-RAHMAN* et al. *1988.* The aw was ranged between 0.81 and 0.94 (Table. 2) and this is favourable for growth of the recorded fungi *DOE and OLLEY, 1992).*

MOTOHIRO, 1988 in Japan found that the first mould affection on dried salted fillets were *Penicillium* and *Aspergillus* while on dried bonito sticks were *A.glaucus, A. melleus, A. flavoviridescens, P. glaucum, Torula* sp., *Cladosporium herbarum* and *Catenularia fuliginea.*

In the present work *Eurotium (E. halophilicum* and *E. niveoglaucum)* could be isolated in low percentage while *WHEELER* et al. *1986* found *Eurotium (E. repens, E. amstelodami, E. chevalieri)* as a prevalent fungi on Indonesian dried salted fish. Many of the fungal species isolated in the current study are pathogenic or toxigenic for human or produce aflatoxins *(Aspergillus flavus),* fumigillin *(A. fumigatus)* nigragillin, malformin and naphthoquinones *(A.niger),* Sterigamatocystin *(Aspergillus sydowii, A. versicolor* and Emericella nidulans) *(FRISVAD, 1988 and NORTHOLT and SOENTORO, 1988).*

The use of halophilic malt extract agar recorded good results for recovery of fungi from salted fish more than the ordinary malt agar, so we recommended the halophilic agar for this purpose.

The count of mould in the present study was in agreement with that reported by *MORSHDY et al. 1982, ABDEL-RAHMAN et al. 1988 and LU et al. 1988.*

The higher fungal contamination of salted fish in this investigation may be attributed to improper cleaning of fish before salting, the use of fish for salting without removing the abdominal viscera, poor quality of the used salt, hygienic of the salting Process and or bad storing conditions. The overcoming of these problems may yeild decrease or prevention of such contamination.

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Table (1): Fungi associated with salted fish

Fungi	MA		HM	
	C	%I	C	%I
Acremonium strictum	7x10	4.4	-	-
Alternaria alternata	-	-	$1.2x10^2$	8.9
Aspergillus	$3.7x10^3$	53.3	$5.3x10^3$	75.6
A. alutaceus	$1.8x10^2$	8.9	$2.1x10^2$	22.6
A. flavus	$1.6x10^3$	31.1	$2.4x10^2$	57.8
A. flavus var. columnaris	-	-	$1.6x10^2$	11.1
A. fumigatus	$7.7x10^2$	17.8	$5.9x10^2$	44.1
A. niger	$1x10^2$	8.9	$1.5x10^3$	44.1
A. sydowii	$8.5x10^2$	8.9	3x10	4.4
A. terreus	$1.5x10^2$	8.9	$3.8x10^2$	26.7
A. ustus	-	-	7	2.2
A. versicolor	13	2.2	7	2.2
Candida albicans	$8.9x10^3$	11.1	-	-
Cladosporium	$6.8x10^2$	6.7	$2.7x10^3$	40
C. cladosporioides	10	4.4	$2.3x10^3$	37.3
C. sphaerospermum	$6.7x10^2$	2.2	$3.3x10^2$	2.2
Emericella nidulans	-	-	13	2.2
Eurotium	$1x10^2$	2.2	10	4.4
E. halophilicum	$1x10^2$	2.2	7	2.2
E. niveoglaucum	-	-	3	2.2
Fennellia flavipes	-	-	3x10	2.2
Geotrichum candidum	13	2.2	-	-
Gibberella fujikuroi	-	-	$4x10^3$	2.2
Paecilomyces lilacinus	-	-	7x10	4.4
Penicillium	$2.9x10^3$	44.4	$2.5x10^3$	68.9
P. chrysogenum	$2.6x10^3$	40	$2.2x10^3$	66.7

Continuous: table (1)

Fungi	MA		HM	
	C	%I	C	%I
P. oxalicum	-	-	$1.4x10^2$	4.4
P. piceum	$6.7x10^2$	2.2	-	-
P. pinophilum	13	4.4	-	-
P. waksmanii	$1.4x10^2$	6.7	$1.7x10^2$	2.2
Rhizopus stolonifer	10	2.2	-	-
Rhodotorula sp.	$2.9x10^2$	4.4	$5.9x10^2$	8.8
Scopulariopsis halophilica	$1.3x10^2$	2.2	$4x10^2$	2.2
Stachybotrys chartarum	20	4.4	-	-
Total	$1.7x10^4$	80	$1.6x10^4$	97.8
Number of genera (16)	11	-	11	-
Number of species (29+1 variety)	21	-	21+1	-
Bacteria	$1.6x10^4$	31.1	$1.7x10^4$	20

MA: malt extract agar media
HM: halophilic malt agar
C : count per gram
%I: percentage of incidence

Table (2): Statisical analysis of pH, a_w and NaCL %

	Min.	Max.	Mean
PH	6.4	7.1	6.6
a_w	0.81	0.94	0.91
NaCL %	6.5	18.9	13