Effect of sodium chloride, nitrate/nitrite and refrigeration on mycological status of salted *Mugil cephalus* fish

Ismail, Hesham[1,2*], Ahmed, Hussein[2] and Youssef, Alaa[2]
[1]Department of Public Health, College of Veterinary Medicine, King Faisal University, 400 Al-Ahsa, 31982, Saudi Arabia.
[2]Department Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.
*Corresponding author: hismail@kfu.edu.sa

Abstract

The aim of the present study was to evaluate the effect of table salt (sodium chloride), nitrate/nitrite mixture and refrigeration on mycological status of laboratory prepared salted fish. Four groups of salted fish (Mugil cephalus) were prepared in the laboratory using 10% table salt, 10% table salt plus refrigeration, 10% table salt plus nitrate/nitrite mixture or 20% table salt. Samples of fish skin from each group were taken at zero, 5, 10, 15, 20 days and ripening point interval and were inoculated on three different media (Dicloran rose Bengal chloramphenicol agar, malt extract medium + 10% NaCl and malt extract medium + 20% NaCl) to be evaluated for their mycological status. The mycological evaluation revealed that a total number of 19 genera, 53 species and one species variety in addition to some unidentified species of yeasts and sterile mycelia were isolated from fish skin samples. Increasing the concentration of table salt used in salting, adding mixture of nitrate/nitrite and refrigeration had inconsistent effects on the total number of fungi recovered on the three used media.

1. INTRODUCTION

Fish considered as one of our most valuable sources of protein food. Worldwide, people obtain about 25% of their animal protein from fish and shellfish. The protein found in fish is of high biological value **(Bahnasawy et al., 2009)**. Traditionally, fish and fish products have been a popular part of the diet in many parts of the world, where it constitutes a major part of the diet in tropical areas including Africa and Far East **(FAO, 1994)**.

Salting of food and particularly salting of fish goes back to ancient times and still in use nowadays even in developed countries. Consuming salted fish is a common tradition in some Mediterranean countries because of its low production cost and favorable palatability **(El-Sebaiy and Metwalli, 1989).**

Fungi widely distributed in nature and could grow over an extremely wide range of temperature. They considered a major factor in the spoilage of fish and fish products that lead

to economic losses. Moulds probably contaminate and spoil more foods, including fish, than any other group of microorganisms. They render contaminated foods not only unpalatable, but also unsafe for consumption by producing mycotoxin. Mycotoxins have been associated with several cases of human poisoning or mycotoxicosis, sometimes resulting in death **(Dvorackova, 1990; Cardwell, 1999).** High mycotoxins levels could lead to liver cancer, whereas subacute levels are responsible for liver disease and organ damage **(Pitt, 2000).**

Using of table salt (Sodium chloride) in fish preservation is effective in retarding the growth of microorganisms **(FAO, 1981),** where it sets off most of the microbial changes produced during the ripening of fish **(Rodriguez-Jerez et al., 1993)**.

Nitrate (NO_3) was added to the curing salt mixture of certain semi-preserved pickled fish products in order to delay spoilage and to control microbial activity during storage **(Pederson and Meyland, 1981; Knøchel and Huss, 1984).** It may also act as a reservoir of nitrite if nitrate-reducing bacteria are present **(Skovgaard, 1992).** Nitrite is an important antimicrobial agent. It has shown to have an inhibitory effect on bacterial spoilage and *Clostridium botulinum* growth and toxin production also in fish **(Sofos *et al.*, 1979; Pierson and Smoot, 1987; Hyytia *et al.*, 1997).**

Refrigerated fish were exposed to spoilage by some strain of moulds such as *Penicillium* (blue and green spots), *Cladosporium herbarum* (black spots), *Mucor and Rhizopus* (whiskers), and *Chyrsosporiztm pannorum* (white spots) **(Ayers, 1963)**. Deep freezing has no significant effect upon mould spores, however they can resist cold temperature up to 40°C.

This study was performed to evaluate the effect of table salt concentration, adding nitrate/nitrite mixture and refrigeration on mycological status of laboratory prepared salted fish.

2. MATERIAL AND METHODS

2.1. Collection of samples

Ten kilograms of fresh *Mugil cephalus* (**Linnaeus, 1758**) fish (each weighing 220-340g and of length ranging from 23-28 cm) were bought from fish sales outlets in Assiut city, Egypt. The samples were transferred to the laboratory in an icebox with a minimum of delay.

2.2. Preparation of samples

At the laboratory, fresh fish was subjected to sensory examination to ensure its freshness then washed under running tap water to remove visible dirt. Fish was divided randomly into four groups. Group 1 salted by using 10% NaCl; group 2 salted by using 10%

NaCl with refrigeration; group 3 salted by using 10% NaCl and Na nitrate (2/3)/Na nitrite (1/3) mixture (100 ppm); group 4 salted by using 20% NaCl. Groups 1, 3 and 4 were preserved at room temperature (25± 2ºC) while only group 2 was stored at refrigerator (4°C) until ripening.

2.3. Salting method

A uniform layer of salt was spread over the bottom of the metal container then the fish were placed in several layers with salt distributed uniformly in between, finishing with the layer of salt placed on the top layer of the fish. The packed fish was covered with a plastic bag and a suitable weight was placed over them to help pressing and fluid extraction. The extruded fluid during the first 2 days of salting was discarded. The fish hold for ripening at room temperature (25±2°C) except for the second group that was hold in a refrigerator at 4°C until ripening.

2.4. Mycological examination

Samples of fish from each group were taken at zero time (fresh fish), 5, 10, 15, 20 days and ripening point (40 days except for the second group, which was analyzed at 60 days) interval after salting

The fish heads, scales, tails, fins, guts and bones were removed and discarded. All fish skin from head to tail and from top of back to belly on both sides were obtained and thoroughly homogenized in a sterile mortar and used for mycological examination.

Skin samples were prepared according to the technique recommended by **American Public Health Association "APHA" (1985).** To 10 grams of skin, 90 ml of sterile saline solution (0.85% w/v) were added aseptically and thoroughly mixed for not more than 2.5 minutes, to avoid mycelial fragment, using a sterile warning blender. Such homogenate represents the dilution of 10^{-1}. The homogenized sample was mixed by shaking and 10 ml of the original dilutions was transferred into sterile flask containing 90 of sterile saline solution and mixed carefully by shaking. Several dilutions were done in a sequential manner by tenfold serial dilution to obtain suitable number of colonies that could be easily counted.

Three types of media were used for the isolation and enumeration of fungi: dicloran rose-bengal chloramphenicol agar medium, DRBC **(King et al., 1979),** malt extract medium + 10% NaC1 (MSA10%) and malt extract medium + 20% NaC1 (MSA20%) **(Blakeslee, 1915)**. Five plates for each medium type were used and incubated at 25°C for 5-20 days during which the developing colonies were counted, identified and the total mould count/g were calculated. The identification of mould genera and species were carried out on the basis of their macroscopic and microscopic characteristics following the identification keys of **Moubasher (1993); Samson *et al*. (2004); Leslie and Summerell (2006); Domsch *et al.* (2007); Pitt and Hocking (2009).**

3. Results

Table (1): Mean Counts of fungal genera and species recovered from the experimentally prepared salted fish with 10% NaCl on DRBC, MSA 10% and MSA 20% at 25° C (Figures in the table are calculated as colony forming units; CFUs/g sample)*.

	DRBC			MSA 10%			MSA 20%		
	TC	**% TC**	**NCI**	**TC**	**% TC**	**NCI**	**TC**	**% TC**	**NCI**
Acremonium	**100**	**0.43**	**2**						
A. hyalinulum	20	0.09	1						
A. strictum	80	0.34	1						
Alternaria alternata	**60**	**0.26**	**2**				**2**	**0.31**	**1**
Aspergillus	**1020**	**4.34**	**6**	**224**	**6.04**	**6**	**28**	**4.30**	**3**
A. candidus	40	0.17	1	6	0.16	1			
A. niger	600	2.55	3	190	5.12	5			
A. ochraceus	40	0.17	1	8	0.22	3	4	0.61	2
A. sydowii	20	0.09	1	6	0.16	2	10	1.53	1
A. terreus	320	1.36	4	14	0.38	5	10	1.53	1
Aspergillus sp.							4	0.61	1
Byssochlamys spectabilis	**20**	**0.09**	**1**						
Cladosporium	**820**	**3.49**	**6**	**200**	**5.39**	**5**	**32**	**4.91**	**1**
C. cladosporioides	660	2.81	5	194	5.23	4			
C. herbarum	80	0.34	2				24	3.68	1
C. sphaerospermum				6	0.16	1			
Cladosporium sp.	80	0.34	1				8	1.23	1
Emerciella nidulans	**20**	**0.09**	**1**	**4**	**0.11**	**1**			
Eurotium				**24**	**0.65**	**3**	**8**	**1.23**	**3**
E. amstelodami				8	0.22	2	6	0.92	2
E. chevalieri				16	0.43	1	2	0.31	1
Fennellia flavipes	**40**	**0.17**	**1**	**12**	**0.32**	**3**			
***Fusarium* sp.**	**40**	**0.17**	**2**						
Geotrichum candidum	**20**	**0.09**	**1**						
Myrothecium roridum	**20**	**0.09**	**1**						
Neosartorya fumigata	**20**	**0.09**	**1**				**14**	**2.15**	**3**
Paecillium lilacinum	**20**	**0.09**	**1**						
Penicillium	**1120**	**4.77**	**5**	**36**	**0.97**	**5**	**14**	**2.15**	**3**
P. aurantiogriseum	440	1.87	3	16	0.43	3			
P. chrysogeneum	480	2.04	3	2	0.05	1	2	0.31	1
P. duclauxii	80	0.34	2						
P. griseofulvum	20	0.09	1						
P. oxalicum				18	0.49	2			
P. pinophilum	20	0.09	1						
Penicillium sp.	80	0.34	1				12	1.84	2
Scopulariopsis				**2**	**0.05**	**1**	**2**	**0.31**	**1**
S. brumptii							2	0.31	1
S. halophilica				2	0.05	1			
Trichoderma pseudokomingii	**20**	**0.09**	**1**						
Wallemia sebi				**2**	**0.05**	**1**			
Wardomyces columbinus	**180**	**0.77**	**1**						
Yeasts	**19970**	**85.02**	**6**	**3204**	**86.41**	**6**	**552**	**84.66**	**3**
Orange- Red	2740	11.67	2	40	1.08	4			
White	17230	73.35	6	3158	85.17	6	552	84.66	3
Yellow				6	0.16	1			
Total	**23490**	**100**	**6**	**3708**	**100**	**6**	**652**	**100**	**6**
No. of genera/species		**15/27**			**8/16**			**7/14**	

*__TC__: Total counts, __%TC__: Percentage total count (calculated per total counts of all fungi), __NCI__: number of cases of isolation. __DRBC__ = Dicloran Rose Bengal medium, __MSA10%__ = 10 % NaCl malt salt extract agar, __MSA20%__ = 20 % NaCl malt salt extract agar.

Table (2): Mean counts of fungal genera and species recovered from the experimentally prepared salted fish with 10% NaCl and ripening in refrigerator on DRBC, MSA 10% and MSA 20% at 25° C (Figures in the table are calculated as colony forming units; CFUs/g in all samples)*.

	DRBC			MSA 10%			MSA 20%		
	TC	**% TC**	**NCI**	**TC**	**% TC**	**NCI**	**TC**	**% TC**	**NCI**
Acremonium	**160**	**0.63**	**2**						
A. hyalinulum	80	0.32	1						
A. strictum	80	0.32	1						
Alternaria alternata	**40**	**0.16**	**2**				**2**	**0.29**	**1**
Aspergillus	**1460**	**5.75**	**5**	**222**	**8.73**	**6**	**72**	**10.56**	**3**
A. candidus				2	0.08	1	16	2.35	1
A. niger	1240	4.89	5	182	7.16	5			
A. ochraceus	20	0.08	1	8	0.32	2	8	1.17	2
A. sydowii	40	0.16	1	8	0.32	3	10	1.47	1
A. terreus	140	0.55	3	22	0.87	5	4	0.59	2
A. wentii							2	0.29	1
Aspergillus sp.							32	4.69	1
Cladosporium	**740**	**2.92**	**6**	**178**	**7.00**	**5**	**80**	**11.73**	**3**
C. cladosporioides	660	2.60	6	176	6.92	5	56	8.21	3
C. herbarum	20	0.09	1				24	3.52	1
C. sphaerospermum	60	0.24	2	2	0.08	1			
Emerciella nidulans	**40**	**0.16**	**2**	**2**	**0.08**	**1**			
Eurotium	**60**	**0.24**	**1**	**50**	**1.97**	**6**	**42**	**6.16**	**3**
E. amstelodami				20	0.79	2	36	5.28	1
E. chevalieri				24	0.94	4	2	0.29	1
Eurotium sp.	60	0.24	1	6	0.24	1	4	0.59	2
Fennellia flavipes	**20**	**0.08**	**1**	**10**	**0.39**	**3**	**2**	**0.29**	**1**
Fusarium	**80**	**0.32**	**2**	**2**	**0.08**	**1**			
Fusarium sp.	80	0.32	2	2	0.08	1			
Geotrichum candidum	**40**	**0.16**	**2**						
Microascus cinereus	**20**	**0.08**	**1**						
Myrothecium roridum	**20**	**0.08**	**1**						
Neosartorya fumigate	**60**	**0.24**	**1**	**2**	**0.08**	**1**	**6**	**0.88**	**1**
Paecillium lilacinum	**20**	**0.08**	**1**						
Penicillium	**540**	**2.13**	**5**	**86**	**3.38**	**5**	**64**	**9.38**	**3**
P. aurantiogriseum	220	0.87	2	34	1.34	2	2	0.29	1
P. chrysogenum	40	0.16	1						
P. oxalicum				40	1.57	1			
P. puberulum	40	0.16	1						
P. viridicatum	80	0.32	1						
Penicillium sp.	160	0.63	2	12	0.47	3	62	9.09	2
Scopulariopsis							**2**	**0.29**	**1**
S. brumptii							2	0.29	1
Stemphylium botryosum				**2**	**0.08**	**1**			
Trichoderma	**120**	**0.48**	**4**						
T. harzianum	20	0.08	1						
T. pseudokoningii	100	0.35	3						
Wallemia sebi							**2**	**0.29**	**1**
Paecillium lilacinum	**20**	**0.08**	**1**						
Wardomyces columbinus	**180**	**0.71**	**1**						
Yeasts	**21800**	**85.9**	**6**	**1988**	**78.21**	**6**	**410**	**60.11**	**3**
Orange- Red	5740	22.62	6	110	4.33	5			
White	14620	57.60	6	1878	73.88	6	410	60.11	3
Yellow	1440	5.68	4						
Total	**25380**	**100**	**6**	**2542**	**100**	**6**	**682**	**100**	**6**
No. of genera/species		**17/28**		**9/18**			**9/18**		

***As table 1**

Table (3): Mean Counts of fungal genera and species recovered from the experimentally prepared salted fish with 10% NaCl + $NaNO_3$/$NaNO_2$ on DRBC, MSA 10% and MSA 20% at 25°C (Figures in the table are calculated as colony forming units; CFUs/g in all samples)*.

	DRBC			MSA 10%			MSA 20%		
	TC	**% TC**	**NCI**	**TC**	**% TC**	**NCI**	**TC**	**% TC**	**NCI**
Acremonium	**140**	**0.50**	**3**						
A. hyalinulum	60	0.22	2						
A. strictum	80	0.29	1						
Alternaria alternata	**20**	**0.07**	**1**				**2**	**0.13**	**1**
Aspergillus	**358**	**1.28**	**5**	**202**	**12..8**	**5**	**24**	**1.56**	**3**
A. candidus				2	0.13	1			
A. niger	3	0.99	4	180	11.41	4			
A. ochraceus				8	0.51	2	2	0.13	1
A. sydowii	20	0.07	1	4	0.25	1	10	0.65	1
A. tamari				2	0.13	1			
A. terreus	60	0.21	2	6	0.38	2			
Aspergillus sp.							12	0.78	2
Byssochlamys spectabilis	**20**	**0.07**	**1**						
Cladosporium	**870**	**3.11**	**5**	**90**	**5.70**	**5**	**34**	**2.20**	**2**
C. cladosporioides	830	2.97	5	58	3.68	3	10	0.65	2
C. herbarum	20	0.07	1	30	1.90	3	24	1.56	1
C. sphaerospermum	20	0.07	1	2	0.13	1			
Emerciella nidulans	**20**	**0.07**	**1**	**2**	**0.13**	**1**			
Eurotium				**26**	**1.65**	**3**	**4**	**0.26**	**2**
E. amstelodami							2	0.13	1
E. chevalieri				26	1.65	3	2	0.13	1
Fennellia flavipes	**40**	**0.14**	**1**						
***Fusarium* sp.**	**20**	**0.07**	**1**						
Geotrichum candidum	**20**	**0.07**	**1**						
Myrothecium roridum	**20**	**0.07**	**1**						
Neosartorya fumigate				**2**	**0.13**	**1**	**6**	**0.39**	**1**
Paecillium lilacinum	**20**	**0.07**	**1**						
Penicillium	**440**	**1.57**	**5**	**40**	**2.53**	**5**	**16**	**1.04**	**3**
P. aurantiogriseum	280	1.00	4	28	1.78	3			
P. chrysogenum	60	0.22	1	2	0.13	1			
P. oxalicum				2	0.13	1			
P. pinophilum	100	0.36	2						
Penicillium sp.				8	0.51	1	16	1.04	3
Petromyces flavus							**4**	**0.26**	**1**
Scopulariopsis	**20**	**0.07**	**1**				**2**	**0.13**	**1**
S. brumptii							2	0.13	1
S. halophilica	20	0.07	1						
Trichoderma pseudokoningii	**140**	**0.50**	**3**						
Wardomyces columbinus	**180**	**0.64**	**1**						
Yeasts	**25660**	**91.68**	**6**	**1216**	**77.06**	**5**	**1452**	**94.04**	**5**
Orange- Red	1580	5.65	2	206	13.05	2			
White	24080	86.04	6	402	25.48	3	1452	94.04	5
Yellow				608	38.53	3			
Total	**27988**	**100**	**6**	**1578**	**100**	**6**	**1544**	**100**	**6**
No. of genera/species		**15/22**		**6/16**				**8/12**	

*__TC__: Total counts, __%TC__: Percentage total count (calculated per total counts of all fungi), __NCI__: number of cases of isolation. __DRBC__ = Dicloran Rose Bengal medium, __MSA10%__ = 10 % NaCl malt salt extract agar, __MSA20%__ = 20 % NaCl malt salt extract agar.

Table (4): Counts of fungal genera and species recovered from the experimentally prepared salted fish with 20% NaCl on DRBC, MSA 10% and MSA 20% at 25°C. (Figures are calculated as colony forming units; CFUs/g samples)*.

	DRBC			MSA 10%			MSA 20%		
	TC	**% TC**	**NCI**	**TC**	**% TC**	**NCI**	**TC**	**% TC**	**NCI**
Acremonium	**160**	**1.02**	**2**						
A. hyalinulum	80	0.51	1						
A. strictum	80	0.51	1						
Alternaria alternate	**20**	**0.13**	**1**				**2**	**0.29**	**1**
Aspergillus	**860**	**5.51**	**6**	**242**	**10.49**	**6**	**22**	**3.14**	**4**
A. candidus				12	0.52	2	2	0.29	1
A. niger	640	4.10	6	190	8.23	5	2	0.29	1
A. ochraceus				8	0.35	2			
A. sydowii	20	0.13	1	8	0.35	2	2	0.29	1
A. tamarii							10	1.43	
A. terreus	200	1.28	4	24	1.04	5	2	0.29	1
Aspergillus sp.							4	0.57	1
Aureobasidium pullulans							**2**	**0.29**	**1**
Byssochlamys spectabilis	**20**	**0.13**	**1**						
Cladosporium	**1120**	**7.17**	**6**	**268**	**11.61**	**6**	**182**	**26**	**4**
C. cladosporioides	980	6.27	6	252	10.92	6	154	22	4
C. herbarum	20	0.13	1						
C. sphaerospermum	120	0.77	2	16	0.69	1	28	4	2
Emerciella nidulans	**20**	**0.13**	**1**						
Eurotium	**40**	**0.26**	**2**	**22**	**0.95**	**4**	**4**	**0.57**	**1**
E. amstelodami	20	0.13	1	2	0.09	1			
E. chevalieri				20	0.87	3			
E. rubrum							2	0.29	1
Eurotium sp.	20	0.13	1				2	0.29	1
Fennellia flavipes				**2**	**0.09**	**1**			
***Fusarium* sp.**	**20**	**0.13**	**1**						
Geotrichum candidum	**20**	**0.13**	**1**				**6**	**0.86**	**1**
Myrothecium roridum	**20**	**0.13**	**1**						
Nigrospora oryzae							**6**	**0.86**	**1**
Paecillium lilacinum	**20**	**0.13**	**1**						
Penicillium	**1640**	**10.5**	**6**	**72**	**3.12**	**5**	**80**	**11.43**	**4**
P. aurantiogriseum	1040	6.66	3	8	0.35	1	68	9.71	2
P. chrysogenum				2	0.09	1			
P. duclauxii	60	0.38	1						
P. pinophilum	140	0.90	3						
Penicillium sp.	400	2.56	2	62	2.69	3	12	1.71	2
Petromyces flavus	**40**	**0.26**	**2**						
Scopulariopsis	**40**	**0.26**	**1**				**2**	**0.29**	**1**
S. brevicaulis	20	0.13	1						
S. candida	20	0.13	1						
S. halophilica							2	0.29	1
Trichoderma pseudokomingii	**20**	**0.13**	**1**						
Wallemia sebi							**2**	**0.29**	**1**
Wardomyces columbinus	180	1.15	1						
Yeasts	**11360**	**72.72**	**6**	**1702**	**73.74**	**6**	**392**	**56**	**5**
Orange- Red	3040	19.46	6	42	1.82	3			
White	8320	53.27	5	1648	71.40	6	392	56	5
Yellow				12	0.52	3			
Total	**15620**	**100**	**6**	**2308**	**100**	**6**	**700**	**100**	**6**
No. of genera/species		**16/26**			**5/13**			**10/18**	

*__TC__: Total counts, __%TC__: Percentage total count (calculated per total counts of all fungi), __NCI__: number of cases of isolation. __DRBC__ = Dicloran Rose Bengal medium, __MSA10%__ = 10 % NaCl malt salt extract agar.

Table (5): Statistical analytical results of count of fungi on experimental salted fish*.

		ANOVA Sum of Squares	df	Mean Square	F	Sig.
Between Groups		322274.667	3	107424.889	0.605	0.619
DRBC	**Within Groups**	3549146.667	20	177457.333		
Total		3871421.333	23			
Between Groups		5507.167	3	1835.722	0.169	0.916
MSA10%	**Within Groups**	217236.667	20	10861.833		
Total		222743.833	23			
Between Groups		6386.000	3	2128.667	0.727	0.548
MSA20%	**Within Groups**	58589.333	20	2929.467		
Total		64975.333	23			

p>0.05=No sig., p≤0.05=Sig., p≤0.01=H. Sig., p≤0.001=V. H. Sig.

***DRBC** = Dicloran Rose Bengal medium, **MSA10%** = 10 % NaCl malt salt extract agar, **MSA20%** = 20 % NaCl malt salt extract agar.

Table (6): Mean ± Standard Error, Minimum and Maximum of fungal count of experimental salted fish.*

		Minimum	Maximum	Mean ± SE
Salt 10%	**DRBC**	100.00	1420.00	586.67±217.66
	MSA10%	12.00	222.00	84±41.81
	MSA20%	0.00	56.00	16.67±8.59
Salt 10%+Ref	**DRBC**	160.00	1060.00	596.67±155.04
	MSA10%	8.00	236.00	92.33±43.22
	MSA20%	0.00	192.00	45.33±30.54
Salt 10% + ($NaNO_3$/$NaNO_2$)	**DRBC**	100.00	820.00	388±121.39
	MSA10%	8.00	222.00	60.33±33.22
	MSA20%	0.00	56.00	15.33±8.77
Salt 20%	**DRBC**	160.00	1340.00	710±179.31
	MSA10%	16.00	292.00	101±50.21
	MSA20%	6.00	194.00	51.33±29.48

***DRBC** = Dicloran Rose Bengal medium, **MSA10%** = 10 % NaCl malt salt extract agar, **MSA20%** = 20 % NaCl malt salt extract agar.

4. DISCUSSION

Mycological Status of Experimentally Prepared Salted Fish With 10% NaCl and stored at room temperature

The summarized data presented in Table 1 indicated that a total number of 19 genera and 35 species were recovered on the three isolation media from skin parts of the experimentally prepared salted fish with 10 % NaCl. The contamination level was 23499, 3708 and 652 CFU/g on DRBC, MSA10% and MSA20%, respectively.

On dicloran rose-bengal agar, a total number of 15 genera and 27 species in addition to

some unidentified species of red and white yeasts were recorded in the examined skin samples.

Yeasts, *Aspergillus, Cladosporium* and *Penicillium* were isolated in high frequency of occurrence from 6, 6, 6 and 5 sampling times out of the 6 prescribed periods of storage [0 day, 5th day, 10th day, 15th day, 20th day and 40th day (ripening point). They accounted for 85.02, 4.34, 3.49 and 4.77% of the total fungi, respectively.

The most common isolated species were *Aspergillus niger, Cladosporium cladosporioides, Penicillium aurantiogriseum, P. chrysogenum* and *Wardomyces columbinus*, accounting for 2.55%, 2.81%, 1.87%, 2.04% and 0.77% of total fungi, respectively. The other species were recovered in low numbers as *Acremonium strictum, Cladosporium herbarum, Cladosporium sp., Penicillium duclauxii, Penicillium sp.* (0.34% each) *and Alternaria alternate* (0.26%).

On 10% NaCl malt extract media, 8 genera and 16 species in addition to some unidentified species of red, white and yeasts were recorded.

Yeasts, *Aspergillus* and *Cladosporium* were isolated in high frequency of occurrence from 6, 5 and 6 samples, respectively. They constituted 86.41, 6.04 and 5.39% of the total fungi, respectively.

The most common species isolated were, *Aspergillus niger, A. terreus, Cladosporium cladosporioides, Eurotim chevalieri, Penicillium aurantiogriseum* and *P. oxalicum*. They accounted for 5.12, 0.38, 5.23, 0.43, 0.43, 0.49%, respectively, of the total fungi. The other species were recorded in low numbers as *Aspergillus candidus, A. sydowii, C. sphaerospermum* (0.16% each) and *A. ochraceus, E. amstelodami* (0.22% each).

On 20% NaCl malt extract agar, a total number of 7 genera and 14 species in addition to some unidentified white yeasts were recorded. Yeasts, *Aspergillus, Cladosporium, Neosartorya, Penicillium* and *Eurotium* were the common genera recovered on this medium, emerging from 3, 3, 1, 3, 3 and 3 sampling times out of the prescribed periods of storage. They constituted 84.66, 4.30, 4.91, 2.15, 2.15 and 4.46 % of the total fungi.

The most common species isolated from skin part were *Aspergillus sydowii, A. terreus, Cladosporium herbarum, Neosartorya fumigata* and *Penicillium sp*. They accounted for 1.53, 1.53, 3.68, 2.15 and 1.84 % of the total fungi, respectively. The other species were recovered in low numbers and those are *Aspergillus ochraceus* (0.61 %), *Aspergillus* sp. (0.61 %), *Cladosporium* sp. (1.23 %). and *Eurotium amstelodami* (0.92 %).

Mycological Status of Experimentally Prepared Salted Fish With 10% NaCl and Ripened In Refrigerator

The achieved results presented in Table 2 showed that a total numbers of 19 genera and

37 species from the experimentally prepared salted fish with 10 % NaCl and ripened in the refrigerator were recorded.

On DRBC, 17 genera and 28 species in addition to some unidentified species of red-, white- and yellow-colored yeasts were recorded with a total contamination level of 25380 CFU/g.

Yeasts, *Aspergillus, Cladosporium* and *Penicillium* were isolated in high frequency of occurrence from 6, 5, 6 and 5 sampling times out of the 6 prescribed periods of storage [0, 5, 10, 15, 20 and 60 days (ripening point)]. They accounted for 85.9, 5.75, 2.92 and 2.13 % of the total fungi, respectively.

The most common species were Acremonium *hyalinulum, A. strictum, Aspergillus niger, A. terreus, Cladosporium cladosporioides, Fusarium sp., Penicillium aurantiogriseum, P. viridicatum, Penicillium sp., Trichoderma pseudokomingii* and *Wardomyces columbinus*. They accounted for 0.32, 0.32, 4.89, 0.55, 2.60, 0.32, 0.87, 0.32, 0.63, 0.35 and 0.71 %, respectively.

On 10 % NaCl malt extract agar, 9 genera and 18 species in addition to some unidentified species of red and white and yeasts were recorded. The total contamination level was 2542 CFU/g.

Yeasts, *Aspergillus, Cladosporium* and *Penicillium* were isolated in high frequency of occurrence from 6, 6, 5 and 5 sampling times out of the 6 prescribed periods of storage. They constituted 78.21, 8.73, 7.00 and 3.38 % of the total fungi.

The most common species isolated on skin samples were *Aspergillus niger, A. terreus, Cladosporium cladosporioides, Eurotium amstelodami, E. chevalieri, Fennellia flavipes, Penicillium aurantiogriseum, P. oxalicum* and *Penicillium sp*. They accounted for 7.16, 0.87, 6.92, 0.79, 0.94, 0.39, 1.34, 1.57and 0.47% of the total fungi, respectively.

On 20%NaCl malt extract agar, a total number of 9 genera and 18 species in addition to some unidentified species of white yeasts were recorded with a total contamination level of 682 CFU/g.

Yeasts, *Aspergillus, Cladosporium, Eurotium* and *Penicillium* were the common genera recovered on this medium, emerging from 3 sampling times for each out of the 6 prescribed period of storage. They accounted for 60.11, 10.56, 11.73, 6.16 and 9.38 % of the total fungi.

The most common species isolated were *Aspergillus candidus, A. sydowii, Aspergillus sp., Cladosporium cladosporioides, C. herbarum, Eurotium amstelodami* and *Penicillium sp.* They accounted for 2.35, 1.47, 4.69, 8.21, 3.52, 5.28 and 9.09 %, respectively, during the 6 periods of storage. Other species were recovered in low numbers such as *Aspergillus ochraceus*

(1.17 %), *A. terreus*, *Eurotium* sp. (0.59 % each) and *Neosartorya fumigata* (0.88 %).

Mycological Status of Experimentally Prepared Salted Fish With 10% NaCl plus Na Nitrate/Na Nitrite Mixture (100 ppm)

Data in Table 3 showed that a total number of 18 genera and 33 species in addition to some unidentified white, red- and yellow-colored yeasts were recovered on the three isolation media from the experimentally prepared salted fish with 10% NaCl plus $NaNO_3$/$NaNO_2$ mixture (100ppm). The contamination level was the highest on DRBC (27988 CFU/g), followed by 1579 CFU/g on MAS10% and the lowest value was recorded on MAS20% (1544 CFU/g).

On dicloran rose-bengal agar, a total of 15 genera and 22 species in addition to some unidentified species of red- and white-colored yeasts were recorded on the examined samples.

Yeasts, *Aspergillus*, *Cladosporium* and *Penicillium* were isolated in high frequency of occurrence from 6, 5, 5 and 5 sampling times out of the 6 prescribed periods of storage (0, 5, 10, 15, 20th day and ripening point). They accounted for 91.68, 1.28, 3.11 and 1.57 %, respectively.

The most common species isolated from samples are *Acremonium hyalinulum, A. strictum, Aspergillus niger, A. terreus, Cladosporium cladosporioides, Penicillium aurantiogriseum, P. chrysogenum, P. pinophilum Trichoderma pseudokoningii* and *Wardomyces columbinus*. They accounted for 0.22, 0.29, 0.99, 0.21, 2.97, 1.00, 0.22, 0.36, 0.50 and 0.64 % of the total fungi, respectively, during the 6 periods of storage.

On 10%NaCl malt extract media, 6 genera and 16 species in addition to some unidentified species of red, white and yellow yeast were recorded.

Yeasts, *Aspergillus* and *Cladosporium* were isolated in high frequency of occurrence from 5, 5 and 5 sampling times out of the 6 prescribed period of storage. They constituted 77.06, 12.08 and 5.70 % of total fungi, respectively.

The most common species recorded were *Aspergillus niger, Cladosporium cladosporioides, C. herbarum, Eurotium chevalieri* and *Penicillium aurantiogriseum*. They accounted for 11.41, 3.68, 1.90, 1.65and 1.78%, respectively during the 6 periods of storage. Some other species were recovered in low numbers and these are *Aspergillus ochraceus* (0.51 %), *A. sydowii* (0.25 %), *A. terreus* (0.38%) and *Penicillium sp.* (0.51%).

On 20 % NaCl malt extract agar, 8 genera, 12 species and some unidentified yeasts were recorded in skin samples.

Yeasts, *Aspergillus, Cladosporium, Eurotium* and *Penicillium* were the most common

genera on this medium, occurred in 5, 3, 2, 2 and 3 sampling times out of the 6 prescribed periods of storage. They constituted 94.04, 1.56, 2.20, 0.26 and 1.04% of the total fungi, respectively.

The most common species on the examined samples were *Aspergillus sydowii, Aspergillus sp., Cladosporium cladosporioides, Cladosporium herbarum, Neosartorya fumigata* and *Penicillium* sp. They accounted for 0.65, 0.78, 0.65, 1.56, 0.39 and 1.04 % of the total fungi, respectively. Some other species were recovered in low proportions as *Alternaria alternata, Aspergillus ochraceus, Eurotium amstelodami, E. chevalieri, Petromyces flavus* and *Scopulariopsis brumptii* (0.13 % each).

Mycological Status of Experimentally Prepared Salted Fish With 20% NaCl and stored at room temperature

Results presented in Table 4 indicated that a total number of 20 genera and 38 species in addition to some unidentified white-, red- and yellow-colored yeasts were recovered on the three isolation media from skin parts from the experimentally prepared salted fish with 20 % NaCl. The lowest contamination lever was recorded on MSA20% (700 CFU/g) followed by 2308 CFU/g on MSA10% and the highest level was recoded on DCRB (15620 CFU/g).

On DRBC, a total number of 16 genera and 26 species in addition to some un-identified species of red- and white-colored yeasts were recorded.

Yeasts, *Aspergillus, Cladosporium* and *Penicillium* were isolated all in high frequency of occurrence from all the sampling times [0, 5, 10, 15, 20 and 40 days (ripening point)]. They accounted for 72.72, 5.51, 7.17 and 10.50 % of the total fungi, respectively.

The most common species were *Aspergillus niger, A. terreus, Cladosporium cladosporioides, C. sphaerospermum, Penicillium aurantiogriseum, P. pinophilum, Penicillium sp.* and *Wardomyces columbinus*. In the whole period of storage, they accounted for 4.10, 1.28, 6.27, 0.77, 6.66, 0.90, 2.56 and 1.15 %, respectively.

On 10% NaCI malt extract agar**,** 5 genera and 13 species in addition to some unidentified red, white and yellow yeast species were recorded.

Yeasts, *Aspergillus, Cladosporium* and *Penicillium* were isolated in high frequency of occurrence from 6, 6, 6 and 5 sampling times out of the 6 prescribed periods of storage. They constituted 73.74, 10.49, 11.61 and 3.12 % of the total fungi, respectively.

The most common species isolated from skin samples were *Aspergillus niger, A. terreus, Cladosporium cladosporioides, C. sphaerospermum, Eurotium chevalieri* and *Penicillium sp*. They accounted for 8.23, 1.04, 10.92, 0.69, 0.87 and 2.69 % respectively, during the whole periods of storage. Some other species were recovered in low numbers and these are *Aspergillus candidus* (0.52 %) and *A. ochraceus, A. sydowii, Penicillium aurantiogriseum* (0.35 % each).

On 20% NaCI malt extract agar, 10 genera and 18 species were recorded in addition to some unidentified white yeast species.

Yeasts, *Aspergillus Cladosporium* and *Penicillium* were the most frequent genera on this medium, occurred in 5, 4, 4 and 4 sampling times out of the 6 prescribed periods of storage, accounting for 56, 3.14, 26 and 11.43 % of the total fungi, respectively.

The most common species isolated were *Aspergillus tamarii, Cladosporium cladosporioides, C. sphaerospermum, Penicillium aurantiogriseum* and *Penicillium sp.* They accounted for 1.43, 22, 4, 9.71 and 1.71 % of total fungi, respectively. Some other species were recorded in low numbers and these are *Aspergillus* sp. (0.57 %), *Geotrichum candidum* and *Nigrospora oryzae* (0.86 % each).

Statistical analysis of the results indicated that there was non-significant difference between the examined four groups in term of the total number of recovered moulds (Table 5). Numerically, the fish that salted with 10% NaCl and Na nitrate/Na nitrite mixture had the lowest count, followed by the group of fish salted with 10% NaCl and then the group of fish salted with 10% NaCl in refrigerator and the highest count was recovered from the 4th group of fish that was salted with 20% NaCl (Table 6).

5. CONCLUSION

The achieved results of the present study declared that the total number of moulds recovered on malt extract media were less than those recovered on Dicloran Rose – Bengal agar. This difference can be attributed to the inhibitory effect of NaCl (10 or 20 %) added to the medium on growth of many mould strains **(Atapattu and Soarjeova, 1990; Essa, 1998; Ahmed *et al*., 2005).**

Adding a mixture of Na nitrate/Na nitrite (100 ppm) was effective in decreasing the total number of fungi (as recorded on 10 % NaCl malt extract agar) from 3708 to 1578 CFUs/g. While on DRBC, the total number of moulds increased from 23490 to 27988 CFUs/g, and increased from 652 to 1544 CFU/g on 20% NaCl malt extract.

Salting of fish in refrigerator had inconsistent effect on the total number of recorded fungi, where the number decreased from 3708 to 2542 CFU/g on 10% NaCl malt extract agar. On contrary, the number increased from 23490 to 25380 and from 652 to 682 CFU/g on DRBC and 20% NaCl malt extract agar, respectively.

Regarding the salt concentration, increasing the concentration of the salt used in salting from 10 to 20 % was effective in reducing the total number of fungi recovered on DRBC and 10% NaCl malt extract agar from 23490 to 15620 and from 3708 to 2308 CFU/g, respectively. While on 20% NaCl malt extract agar it was not effective where the total number increased from 652 to 700 CFUs/g.

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