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Mycological Evaluation of Imported Frozen Fish

Ibrahim A. Samaha[1], Amr A. Amer[1], Youssef S. Abd-Elshahid[2], Safaa M. El-bialy[2]
[1]Food Hygiene Department, Fac. Vet. Medicine, Alexandria Univ.
[2]Animal Health Research Institute, Alexandria Branch.

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ABSTRACT:

A total number of 100 samples of four types of frozen fish (25 from each of Barbone, Sardine, Baca and Mackerel) were collected from different localities of Alexandria markets. The samples were subjected to mycological examination to evaluate both of yeasts and moulds load of these frozen fish. The result recorded that the predominant genera of the isolated mould from the 4 types of fish were *Asperigellus spp.* and *Penicillium spp.* moulds could be isolated as *Cladosporium spp.*, *Fusarium spp.*, *Alternaria spp.*, *Nigrosporium spp.*, *Paecilomyces spp.*, *Mucor spp.* and *Rhizopus spp.* In addition to other the predominant genus of isolated yeasts, was *Candida spp.*as well as *Torulopsis spp.*, *Rhodotorulla spp. and Geotrichium spp.* This study showed how these types of frozen fish were being contaminated from different sources by yeasts and moulds. Also, the hazardous and public health importance of such contaminants were fully discussed and suggested recommendations to improve its quality and safety were explained.

Corresponding author: Safaa M Elbialy : vet.sm92@yahoo.com

1. INTRODUCTION

Fish and shellfish are one of the most important sources of animal protein and have been widely accepted as a good protein source and other elements for the maintenance of healthy body (Ranvichandran *et al*., 2012). They also provide a good source of high quality protein and contain many vitamins and minerals. Fish are extremely perishable commodity and quality loss can occur very rapidly after catch (Khan and Khan, 2001; Dewi *et al*., 2011). Fungal contamination of fish is considered as the main cause of signs of spoilage as off flavor and unpalatable taste and it may constitute a public health hazard as well as many of economic losses (Hassan *et al*., 2007 and El Ahl, 2010). The presence of these fungi in the stock fish samples might probably make the consumption of them constitute a hazardous to health as similarly because they might contain metabolites produced by the fungi (Adebayo-Tayo *et al*., 2008).

Disease outbreaks due to the consumption of contaminated food and food stuff are a recurring problem worldwide. The major factor contributing to contamination are microorganisms, especially fungi, which produce low-molecular-weight compounds as secondary metabolites, with confirmed toxic properties referred to as mycotoxins. Several mycotoxins reported to date are cosmopolitan in distribution and incur severe health-associated risks including cancer and neurological disorders (Bhat *et al*., 2010). Fungal contaminants found in fish, which were known to cause disease in humans include: *Aspergillus spp.* which produce aflatoxins that causes hepatoma (cancer of the liver), acute hepatitis, reduced red blood cell and decrease immune system in man; *Fusarium sp*. which was reported to produce fumonisin toxin and; *Penicillium spp*. which produces penicillic acid (Wogu and Iyayi, 2011).

2. MATERIAL AND METHODS

A total number of 100 samples of four types of frozen fish (25 from each of Barbone, Sardine, Baca and Mackerel) were collected from different Alexandria markets. Samples were subjected to mycological examination; analyses were performed in the laboratory of Animal Health Research Institute, Alexandria Branch.

1. Mycological examination

1.1. Preparation of samples:

Ten grams of the fish muscles were taken aseptically and mixed with 90 ml of 0.1% sterile buffered peptone water (Oxoid) in a

sterile blender jar for 2-5 minutes to be homogenized. Then, 1 ml of this homogenate was transferred to separate tube containing 9 ml of sterile peptone water from which the tenth fold serial dilution were prepared (APHA, 1985)

1.2. Identification of the isolated mould:

All positive mould cultures were purified by sub-culturing on Sabouraud's dextrose agar (SDA) plates and incubated at 25-28°C for 3-5 days then examined by:

1.2.1. Macroscopically examination: according to the methods of *Larone (1976); Frey et al. (1979) and Samson et al. (1995).*

1.2.2. Microscopical examination: according to *Dvorak and Atanasek 1969.*

1.3. Identification of the isolated yeast:

This was carried out according to the methods of ***Lodder et al., 1970; Kreger Van-Rij, 1984*** *and* ***Koneman et al., 1992*** using the following tests:

1- Growth on Sabouraud′s agar.
2- Ascospore formation.
3- Vegetative reproduction was done according to Rhode et al., 1980 and Feinegold and Martin, 1982.
4- Germ tube test were carried out by Harrigan and McCance, 1976.
5- Sugar fermentation.
6- Sugar assimilation test.

3. RESULTS AND DISCUSSION

Table (1): Incidence of mould in different imported frozen fish species (no:25 of each)

Imported Frozen Fish species	Mould positive samples				
	No.	%	Min.	Max.	Mean ±S.E.M.
Barbone	17	68	1.0×10^3	8.0×10^3	$2.76 \times 10^3 \pm 4.6 \times 10^2$
Sardine	16	64	1.0×10^3	6.0×10^3	$2.93 \times 10^3 \pm 4.0 \times 10^2$
Basa	20	80	2.0×10^3	6.0×10^3	$3.05 \times 10^3 \pm 2.1 \times 10^2$

Table (2): Incidence of yeast in different imported frozen fish species (no: 25 of each)

Imported Frozen Fish species	Yeast positive samples				
	No.	%	Min.	Max.	Mean ±S.E.M.
Barbone	12	48	1.0×10^3	2.02×10^5	$4.46 \times 10^4 \pm 1.67 \times 10^4$
Sardine	14	56	3.0×10^3	1.0×10^5	$3.67 \times 10^4 \pm 8.64 \times 10^3$
Basa	16	64	1.0×10^3	2.62×10^5	$5.22 \times 10^4 \pm 1.80 \times 10^4$
Mackerel	10	40	1.0×10^3	1.64×10^5	$4.60 \times 10^4 \pm 1.67 \times 10^4$

Table (3): Incidence of mould species isolated from different imported frozen fish species.

Mould Species		No. of examined fish samples							
		Barbone (25)		Sardine (25)		Baca (25)		Mackerel (25)	
		No.	%	No.	%	No.	%	No.	%
Asperigellus Species	*A. Flavus*	5	20	6	24	7	28	0	0
	A. fumigatus	4	16	4	16	3	12	0	0
	A. niger	6	24	8	32	9	36	12	48
	A. Terrus	0	0	2	8	3	12	0	0
	A. Ochraceus	0	0	0	0	1	4	0	0
Penicillium sp.		12	48	11	44	14	56	0	0
Fusarium sp.		3	12	4	16	6	24	0	0
Derma taceus species	*Alternaria alternate*	3	12	3	12	4	16	0	0
	Cladosporium sp.	8	32	4	16	2	8	0	0
	Nigrosporium sp.	2	8	6	24	1	4	0	0
Paecilomyces sp.		0	0	0	0	2	8	0	0
orac eus fung	*Mucor sp.*	1	4	1	4	5	20	0	0
	Rhizopus sp.	2	8	0	0	3	12	0	0

Table (4): Incidence of yeast species isolated from different imported frozen fish species.

Mould Species	No. of examined fish samples							
	Barbone (25)		Sardine (25)		Basa (25)		Mackerel (25)	
	No.	%	No.	%	No.	%	No.	%
Candida sp.	9	36	10	40	12	48	8	32
Torulopsis sp.	5	20	3	12	5	20	0	0
Rhodotorulla sp.	0	0	6	24	0	0	0	0
Geotrichium sp.	3	12	5	20	7	28	2	8

Recorded result in table (1) shows that 68% of 25 examined imported frozen barbone samples were contaminated with mould and the minimum mould count was 1.0×10^3, the maximum mould count was 8.0×10^3 with a mean value of $2.76 \times 10^3 \pm 4.6 \times 10^2$ cfu /g. Also excluded that 64% of 25 examined imported frozen sardine samples was positive for the presence of mould, the minimum mould count was 1.0×10^3 and the maximum mould count was 6.0×10^3 with a mean value of $2.93 \times 10^3 \pm 4.0 \times 10^2$ cfu/g. Results also shows that 80% of 25 examined imported frozen basa samples were contaminated with mould, the minimum mould count was 2.0×10^3 and the maximum mould count was 6.0×10^3 with a mean value of $3.05 \times 10^3 \pm 2.1 \times 10^2$ cfu/g. While there was 48% mould positive of 25 imported frozen mackerel samples, the minimum mould count was 1.0×10^3 and the maximum mould count was 7.0×10^3 with a mean value of $2.83 \times 10^3 \pm 5.5 \times 10^2$ cfu/g. Regarding table (1); data showed that the higher mould positive samples was obtained from Basa fish (80%) followed by Barbone fish (68%), Sardine fish (64%) then Mackerel fish (48%).Table (2), shows that the percent of yeast positive samples were 48% of examined 25 samples of imported frozen barbone and the minimum, maximum and mean value of total yeast count were 1.0×10^3, 2.02×10^5 and $4.46 \times 10^4 \pm 1.67 \times 10^4$ cfu/g, respectively. It also shows that the percent of yeast positive samples of examined 25 samples of imported frozen sardine was 65% with a minimum, a maximum and a mean value of total yeast count of 3.0×10^3, 1.0×10^5 and $3.67 \times 10^4 \pm 8.64 \times 10^3$ cfu/g, respectively.

It also posed that the yeast positive samples of examined 25 samples of imported frozen Baca was 64% and the minimum, maximum and mean value of total yeast count were 1.0 x 10^3, 2.62 x 10^5 and 5.22 x 10^4± 1.80 x 10^4 cfu/g, respectively. While the percent of yeast positive samples of examined 25 samples of imported frozen mackerel was 40% and the minimum, maximum and mean value of total yeast count were 1.0 x 10^3, 1.64 x 10^5 and 4.60 x 10^4± 1.67 x 10^4 cfu/g. Recorded results in table (2) also showed that the higher yeast positive samples obtained from Baca (64%) followed by Sardine (56%) then Barbone (48%) then Mackerel (40%). This result nearly similar to (Junaid, et al. 2010) findings that (100%) of stock fish samples obtained were contaminated with fungi. these high percentage may be due to that fish has low moisture content which make it more susceptible to fungi action than bacteria (Holdsworth, 1971). This is also in agreement with the findings of (Eaton and Groopman, 1994) that moulds have the ability to survive harsh conditions and low moisture content (Ekundayo, 1984). Also this result may be due to that very often the fish are displayed in open baskets or on tables beside the gutter or refuse dumps and this encourages fungi attack and subsequent production of toxins. On the other hand, data in table (3) revealed that the numbers and percent of predominant genera of the isolated mould from the imported frozen barbone were *Penicillium spp.* 12 (48%) followed by *Cladosporium spp*. 8 (32%), *Asperigellus niger* 6 (24%), *Asperigellus flavus* 5 (20%), *Asperigellus fumigates* 4(16%), *Fusarium* and *Alternaria alternate* 3 (12%), *Nigrosporium spp.* and *Rhizopus spp*. 2 (8%) and *Mucor spp.* 1(4%) . It also revealed that the predominant genera of the isolated mould from the imported frozen sardine were *Penicillium spp.* 11 (44%) followed by *Asperigellus niger* 8 (32%), *Asperigellus flavus* and *Nigrosporium spp.* 6 (24%), *Asperigellus fumigatus* 4 (16%), *Cladosporium spp.* and *Asperigellus Terrus* 2 (8%) and *Mucor spp.* 1 (4%). Results in table (3) also revealed that the predominant genera of the isolated mould from the imported frozen Baca were *Penicillium spp.* 14 (56%) followed by *Asperigellus niger* 9 (36%) , *Asperigellus flavus* 7 (28%) , *Fusarium spp.* 6 (24%) , *Mucor spp.* 5 (20%) , *Alternaria alternate* 4 (16%) , *Asperigellus fumigatus, Asperigellus terreus* and *Rhizopus spp.* 3 (12%) , *Cladosporium spp.* and *Paecilomyces spp.* 2 (8%) , *Asperigellus Ochraceus* and *Nigrosporium spp.* 1 (4%). While the only genus which isolated from the mackerel samples was *Asperigellus niger* 12 (48%). Also table (4) revealed that the predominant genera of the isolated yeast from imported frozen barbone samples was *Candida spp.* 9 (36%) followed by *Torulopsis spp.* 5 (20%), *Geotrichium spp.* 3 (12%). Also, the predominant genera of the isolated yeast from imported frozen sardine samples was *Candida spp.* 10 (40%) followed by *Rhodotorulla spp.* 6 (24%), *Geotrichium spp* 5 (20%) and *Torulopsis spp.*3 (12%). It also shows that the predominant genera of the isolated yeast from imported frozen Baca samples was *Candida spp.* 12 (48%) then *Geotrichium spp.* 7 (28%) and *Torulopsis spp.* 5 (20%). While the predominant genera of the isolated yeast from the imported frozen mackerel samples was *Candida spp.* 8 (32%) followed by *Geotrichium spp.* 2 (8%).Recorded data in tables (3 and 4) also showed that the species of mould and yeast isolated from Barbone were *Asperigellus Flavus, A.fumigatus , Penicillium spp., Fusariumspp., Alternaria sp., Cladosporium sp., Nigrosporium sp., Mucor sp., Rhizopus sp., Candida sp., Torulopsis sp., Geotrichium spp* .and which isolated from Sardine were *Asperigellus Flavus, A.fumigatus , A.niger, A.terrus, Penicilliumsp., Fusariumspp., Alternaria sp., Cladosporium sp., Nigrosporium sp., Mucor sp., Candida sp., Torulopsis sp., Rhodotorulla sp., Geotrichium sp.* And species of Baca were *Asperigellus Flavus, A.fumigatus, A.niger, A.terrus, A.ochraceus, Penicillium spp., Fusariumspp. , Alternaria sp., Cladosporium sp., Nigrosporium spp., Paecilomyces sp., Mucor spp., Rhizopus sp., Candida sp., Torulopsis sp., Geotrichium spp.*While in Mackerel the only mould species isolated was *Asperigellus niger* and the yeast species were *Candida sp.* and *Geotrichium spp.* The isolation of these organisms in fish is in tandem with the reports of (Refai *et al*., 2010; Junaid *et al*., 2010; Iqbal *et al*., 2012 and Iqbal and Mumtaz, 2013). The presence of these fungi in fish could be associated to contamination from the environment, the personnel, water and utensils (Geldreich and Clarke, 1966 and Adams and Moss, 2000).

5. CONCLUSION

Therefore our study poses that the examined frozen fish samples were highly contaminated with different species of mould and yeast; these indicate poor hygienic conditions and lake of hygienic awareness's among frozen fish handlers and processors. So, we strictly recommended that, the processors/handlers/sellers should apply strict hygienic measures so they will not serve as sources for mould and yeast as well as other microbial contamination of these frozen seafood products.

Also, we suggested that processors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of microbial contamination. In addition caution should be taken by consumers in preparation and applying perfect cooking in consuming frozen fish to be safe for consumption.

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