Bacteriological Evaluation of Some Fresh and Frozen fish

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A B S T R A C T

Ninety random samples of fresh and frozen each of *Tilapia niloticus, Mugil cephalus* and Shrimp (15 of each) were collected from different fish markets at Qaliuobia Governorate. All collected samples were bacteriologically examined for determination of Aerobic Plate Count (APC), psychrotrophic count, Isolation and Identification of *pseudomonas* and *Aeromonas* species. The bacteriological examination revealed the mean values of APC in the examined fish samples was $1.52\times10^6 \pm 0.47\times10^6$ and $3.08\times10^5 \pm 1.31\times10^5$ in fresh and frozen *Tilapia niloticus*, $7.14\times10^5 \pm 1.84\times10^5$ and $2.59\times10^5 \pm 0.62\times10^5$ in fresh and frozen *Mugil cephalus* and $7.81\times10^4 \pm 2.07\times10^4$ and $1.36\times10^4 \pm 0.42\times10^4$/g, in fresh and frozen shrimp . The mean values of psychrotrophic count in fresh and frozen Tilapia niloticus were $2.67\times10^5 \pm 0.45\times10^5$ and $5.36\times10^5 \pm 1.24\times10^5$ cfu/g, respectively. $4.91\times10^4 \pm 0.93\times10^5$ and $1.72\times10^5 \pm 0.46\times10^5$/ gm respectively in fresh and frozen Mugil *cephalus*. $7.81\times10^4 \pm 2.07\times10^4$ and $6.51\times10^4 \pm 1.80\times10^4$/g, in fresh and frozen shrimp. The incidence of *Pseudomonas* species was 33.3% and 53.3%in the examined samples of fresh and frozen Tilapia niloticus, 46.7% and 60.0 %in fresh and frozen *Mugil cephalu*, respectively, 33.3 %and 40.0 %in fresh and frozen shrimp, respectively. The incidence of *Aeromonas* species were 46.7 %and 33.3 %in the examined samples of fresh and frozen Tilapia niloticus, 53.3 % and 40.0% in fresh and frozen *Mugil cephalus*, respectively, 33.3 % and 40.0 %in fresh and frozen shrimp, respectively.

Key words: Fresh fish, Frozen fish, Tilapia niloticus, *Mugil cephalus*

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1. Introduction

Fish is an important food with high nutritive value, scince it is a source of low price animal protein and other important nutrients such as calcium, phosphorus, and vitamins. Fresh fish is extremely perishable and the time elapsed post catch and the holding temperature significantly affect the odor and flavor profile of the fish (Russell, 1998). Off odor and off flavor are produced when bacterial numbers reach 10^7 cfu /g of fish (Jay, 2000). Bacterial contamination is either due to direct contamination of the fish by polluted water or due to secondary contamination during handling, processing, storage, distribution or preparation. Such contamination is of particular importance when fish is eaten raw or only lightly processed. The most bacterial species responsible for fish spoilage are *Pseudomonas* and *Aeromonas (Lu and Bi, 2007)*. Aerobic plate count on fish generally do not relate to food safety hazards, but sometimes can be useful to indicate quality, shelf life and post heat processing contamination. Fresh fish and fishery products often have an APC of 10^6 - 10^8 without objectionable quality changes (Nickelson and Finne, 1992). Psychrotrophs are these bacteria that grow well at or below 7° C and have their optimum temperature for growth between 20-30° C. Some psychrotrophic pathogens can grow in the refrigerated food with little or no obvious change of sensory characteristics (Berrang et al., 1989). *Pseudomonas* species are important spoilage organisms in many chilled food products especially fish in which they become the dominant microflora during chill storage (Gram, 1993). In addition, their presence in fish create a great risk as they lead to poisoning and / or spoilage of fish (Jay, 2000). *Aeromonas* species play a major role in the spoilage of fresh fish and its products (Gram and Huss, 1996). Also, they are responsible for wide range spectrum of diseases among aquatic organisms and human (Ebanks et al., 2005) as motile *Aeromonas Septicemia* in fish which is caused by *A. hydrophila* leading to high mortalities and high economic losses (Dhanaraj et al., 2008). Therefore, the present work was planned out to determine the Aerobic Plate Count and Total psychrotrophic count of some Fresh and frozen fish (Tilapia niloticus, Mugil cephalus and Shrimp) and isolation and identification of *pseudomonas* and *Aeromonas* species.

2. MATERIAL AND METHODS

2.1. Collection of fish samples:

A total of 90 random samples of fresh and frozen of each of *Tilapia niloticus, Mugil cephalus* and Shrimp (15 of each) were collected from different fish markets at Kaliobia Governorate. Each sample were kept individually in separate plastic bag and transferred directly to the laboratory in an insulated ice box under complete aseptic conditions without undue delay. All collected samples were examined bacteriologically as rapidly as possible.

2.2. Preparation of samples (APHA, 1992.):

Accurately, 25 grams of the examined fish flesh were transferred to a sterile polyethylene bag and 225 ml of 0.1% sterile buffered peptone water were aseptically added to the content of the bag. Each sample was then homogenized in a blender at 2000 rpm for 1-2 minutes to provide a homogenate of 1/'10 dilution from which ten fold serial dilutions were prepared.

2.2.1. Aerobic plate count (FAO., 1992).

One ml from each of the previously prepared serial dilutions was carefully transferred into each of the appropriately marked sterile petri-dishes, and thoroughly mixed with about 15 ml of previously melted and cooled (45±1°C) standard plate count agar. After solidification, the inoculated plates were inverted and incubated at 35° C for 48hr.Plates with 25-250 colonies were selected and counted.

2.2.2. Determination of Psychotropic count (Collins and Lyne, 1984).

One ml from each of the previously prepared serial dilutions, was transferred by using a sterile pipette into two separate sterile Petri-dishes to which approximately 15 ml of sterile melted and tempered plate count agar (45°C) were added and mixed. The inoculated plates were gently shaken in rotatory movement and left till complete solidification of the agar. The plates were inverted and incubated at 7°C for 10 days. The total Psychrotrophic count/g was calculated on plates containing 30-300 colonies.

2.2.3. Isolation of Pseudomonas species (ICMSF., 1996): Pseudomonas Aeromonas agar medium.

Identification of Pseudomonas species, (Krieg and Holt, 1984). Microscopical examination (A.P.H.A., 1992.). Biochemical identification: (Quinn et al., 2002). Isolation of Aeromonas species (ICMSF., 1996): Pseudomonas Aeromonas agar medium. Identification of Aeromonas species: Microscopical examination (A.P.H.A., 1992.). Biochemical identification: (Baron and Finegold, 1990).

3. RESULTS

Regarding the results recorded in table (1), it is obvious that the APC (cfu/g) of each of fresh *Tilapia niloticus*, fresh *Mugil cephalous*, fresh shrimp, frozen *Tilapia niloticus*, frozen *Mugil cephalous* and frozen shrimp were $1.52\times10^6 \pm 0.47\times10^6$, $7.14\times10^5 \pm 1.84\times10^5$, $7.81\times10^4 \pm 2.07\times10^4$, $3.08\times10^5 \pm 1.31\times10^5$, $2.59\times10^5 \pm 0.62\times10^5$ and $1.36\times10^4 \pm 0.42\times10^4$ respectively. The psychrotrophic count/ g of the examined samples of each of fresh *Tilapia niloticus*, fresh *Mugil cephalous*, fresh shrimp, frozen *Tilapia niloticus*, frozen *Mugil cephalous* and frozen shrimp recorded in table (2) were $2.67\times10^5 \pm 0.45\times10^{5,}$ $6.51\times10^4 \pm 1.80\times10^{4,}$ $2.88\times10^4 \pm 0.59\times10^4$, $5.36\times10^5 \pm 1.24\times10^5$, $1.72\times10^5 \pm 0.46\times10^5$ and $4.91\times10^4 \pm 0.93\times10^5$ respectively. Incidence of identified psychrotrophic bacteria isolated from the examined samples of fish was shown in table (3). Accurately, *Acinetobacter lwoffii, Acinetobacter baumanii, Acinetobacter calcoaceticus, Alcaligenes latus, Alcaligenes faecalis, Flavobacterium aquatile, Moraxella saccharolytica, Neisseria elongate and Neisseria Lactamica.* The incidence of *Pseudomonas* species was 33.3% and 53.3%in the examined samples of fresh and frozen Tilapia niloticus, 46.7% and 60.0 %in fresh and frozen *Mugil cephalus*, respectively, 33.3 %and 40.0 %in fresh and frozen shrimp, respectively table (4). The incidence of *Aeromonas* species were 46.7 %and 33.3 %in the examined samples of fresh and frozen Tilapia niloticus, 53.3 % and 40.0% in fresh and frozen *Mugil cephalus*, respectively, 33.3 % and 40.0 %in fresh and frozen shrimp respectively (table 6).

4. DISCUSSION

Aerobic plate count on fish s generally do not relate to food safety hazards, but sometimes can be useful to indicate quality, shelf life and post heat processing contamination. Fresh fish and fishery products often have an APC of 10^6 - 10^8 without objectionable quality changes (Nickelson and Finne, 1992). The APC of the examined samples of each of fresh *Tilapia niloticus*, fresh *Mugil cephalous*, fresh shrimp, frozen *Tilapia niloticus*, frozen *Mugil cephalous* and frozen shrimp recorded in table (1) were $1.52\times10^6 \pm 0.47\times10^6$, Table (1): Analytical results of (APC) /g of the examined fish samples (n=15).

Fish status	Min.	Max.	Mean ± S.E*
A. Fresh fish:			
Tilapia niloticus	2.8×10^{3}	9.6×10^{6}	$1.52\times10^{6} \pm 0.47\times10^{6}$
Mugil cephalus	4.7×10^{3}	8.5×10^{6}	$7.14\times10^{5} \pm 1.84\times10^{5}$
shrimp	5.2×0^{2}	8.7×10^{5}	$7.81\times10^{4} \pm 2.07\times10^{4}$
A. Frozen fish:			
Tilapia niloticus	3.7×10^{3}	7.6×10^{6}	$3.08\times10^{5} \pm 1.31\times10^{5}$
Mugil cephalus	1.9×10^{3}	4.8×10^{6}	$2.59\times10^{5} \pm 0.62\times10^{5}$
shrimp	1.3×10^{2}	9.6×10^{5}	$1.36\times10^{4} \pm 0.42\times10^{4}$

S.E*= Standard error

Table (2): Analytical results of psychrotrophic count/g of the examined fish samples (n=15).

Fish status	Min.	Max.	Mean ± S.E*
A. Fresh fish:			
Tilapia niloticus	4.9×10^{3}	3.5×10^{6}	$2.67\times10^{5} \pm 0.45\times10^{5}$
Mugil cephalus	1.5×10^{3}	7.9×10^{5}	$4.91\times10^{4} \pm 0.93\times10^{5}$
shrimp	1.3×10^{2}	1.8×10^{4}	$2.88\times10^{4} \pm 0.59\times10^{4}$
A. Frozen fish:			
Tilapia niloticus	7.5×10^{3}	5.8×10^{6}	$5.36\times10^{5} \pm 1.24\times10^{5}$
Mugil cephalus	3.7×10^{3}	1.9×10^{6}	$1.72\times10^{5} \pm 0.46\times10^{5}$
shrimp	2.6×0^{2}	4.3×10^{4}	$6.51\times10^{4} \pm 1.80\times10^{4}$

S.E*= Standard error

Table (3): Serotyping of psychrotrophes isolated from the examined samples of fresh and frozen fish

Serotypes	Fresh fish						Frozen fish					
	Tilapia		Mugil cephalus		Shrimp		Tilapia		Mugil cephalus		Shrimp	
	No	%	No	%	No	%	No	%	No	%	No	%
Acinetobacter lwoffii	12	80	11	73.3	7	46.6	14	93.3	10	66.6	10	66.6
Acinetobacter baumanii	7	46.6	10	66.6	-	-	-	-	11	73.3	10	66.6
Acinetobacter calcoaceticus	6	40	7	46.6	7	46.6	10	66.6	8	53.3	9	60
Alcaligenes latus	12	80	-	-	8	53.3	13	86.6	-	-	-	-
Alcaligenes faecalis	9	60	13	86.6	9	60	14	93.3	15	100	12	80
Flavobacterium aquatile	7	46.6	6	40	6	40	-	-	7	46.6	6	40
Moraxella saccharolytica	4	26.6	3	20	3	20	3	20	4	26.6	-	-
Neisseria elongata	-	-	-	-	-	-	6	40	-	-	-	-
Neisseria Lactamica	3	20	-	-	-	-	-	-	-	-	3	20

% was calculated according to total number of samples (15)

Table (4): Incidence of pseudomonas species isolated from the examined samples of fresh and frozen fish (n=15)

Samples	No. of +ve samples	% of + ve samples
Fresh Tilapia niloticus	5	33.3
Fresh Mugil cephalus	7	46.7
Fresh shrimp	5	33.3
Frozen Tilapia niloticus	8	53.3
Frozen Mugil cephalus	9	60.0
Frozen shrimp	6	40.0

Table (5): Serotyping of pseudomonas species isolated from the examined samples of fresh and frozen fish

Serotypes	Fresh fish						Frozen fish					
	Tilapia		Mugil cephalus		shrimp		Tilapia		Mugil cephalus		Shrimp	
	No	%	No	%	No	%	No	%	No	%	No	%
Pseudomonas Aeruginosa	-	-	-	-	5	33.3	-	-	-	-	4	26.6
Pseudomonas fluorescens	5	33.3	7	46.6	5	33.3	8	53.3	9	60	6	40
Pseudomonas alcaligenes	5	33.3	6	40	-	-	8	53.3	9	60	6	40
Pseudomonas putrefaciens	3	20	7	46.6	5	33.3	6	40	9	60	-	-
Pseudomonas fragi	3	20	3	20	4	26.6	-	-	-	-	3	20
Pseudomonas acidoverans	2	13.3	3	20	-	-	-	-	5	33.3	-	-
Pseudomonas vesicularis	2	13.3	-	-	3	20	-	-	-	-	-	-
Pseudomonas proteolytica	1	6.66	4	26.6	-	-	-	-	-	-	5	33.3
Pseudomonas pseudoalcaligenes	-	-	2	13.3	3	20	-	-	-	-	3	20
Pseudomonas cepacia	-	-	1	6.66	2	13.3	-	-	2	13.3	2	13.3
Pseudomonas diminuta	-	-	-	-	2	13.3	3	20	3	20	-	-
Pseudomonas putida	-	-	-	-	-	-	3	20	-	-	2	13.3

% was calculated according to total number of samples (15)

Table (6): Incidence of *Aeromonas* species isolated from the examined samples of fresh and frozen fish (n=15)

samples	No. of +ve samples	% of + ve samples
Fresh Tilapia niloticus	7	46.7
Fresh Mugil cephalus	8	53.3
Fresh shrimp	5	33.3
Frozen Tilapia niloticus	5	33.3
Frozen Mugil cephalus	6	40.0
Frozen shrimp	6	40.0

Table (7): Serotyping of *Aeromonas* species isolated from the examined samples of fresh and frozen fish

Serotypes	Fresh fish						Frozen fish					
	Tilapia		Mugil cephalus		shrimp		Tilapia		Mugil cephalus		shrimp	
	No	%	No	%	No	%	No	%	No	%	No	%
Aeromonas hydrophila	7	46.6	8	53.3	5	33.3	5	33.3	6	40	-	-
Aeromonas punctate	7	46.6	8	53.3	3	20	5	33.3	6	40	5	33.3
Aeromonas diversa	6	40	6	40	4	26.6	4	26.6	5	33.3	6	40
Aeromonas veronii	5	33.3	4	26.6	-	-	-	-	4	26.6	5	33.3
Aeromonas bestirum	4	26.6	-	-	-	-	-	-	3	20	-	-
Aeromonas fluvialis	-	-	-	-	3	20	-	-	3	20	4	26.6

$7.14\times10^5 \pm 1.84\times10^5$, $7.81\times10^4 \pm 2.07\times10^4$, $3.08\times10^5 \pm 1.31\times10^5$, $2.59\times10^5 \pm 0.62\times10^5$ and $1.36\times10^4 \pm 0.42\times10^4$ respectively. Higher counts of APC values were obtained by (Hytham, 2005) who found that the mean values for sardine ($3.95x10^6$), mullus spp ($3.67\ x10^6$), pargus spp. ($3.82\ x10^6$) and for chrysophyres ($2.16\ x10^6$). Lower results of APC values were obtained by (Shetty and Setty, 1990) who studied the bacteriological status of indian oil sardine stored in chilled sea water and found that the intial total plate count of fresh fish was $3.6x10^3$/g which increased to $8.1x10^3$/g during storage in chilled sea water (2±1° C).

The psychrotrophic bacteria have received an increased attention by several investigators during recent years because fish held for long period at low temperature which greatly slow the multiplication of bacteria, but not stop their growth. Therefore, the favorable conditions for growth of psychrotrophic occurred. A rapid continuous gradual increase in psychrotrophic bacteria during storage of frozen fish can be occured (Al- Habib and Al- Aswad, 1986). The results of psychrotrophic count (cfu/ g) of the examined samples of each of fresh *Tilapia niloticus*, fresh *Mugil cephalous,* fresh shrimp, frozen *Tilapia niloticus,* frozen *Mugil cephalous* and frozen shrimp recorded in table (2) were $2.67\times10^5 \pm 0.45\times10^{5,}$ $6.51\times10^4 \pm 1.80\times10^{4,}$ $2.88\times10^4 \pm 0.59\times10^4$, $5.36\times10^5 \pm 1.24\times10^5$, $1.72\times10^5 \pm 0.46\times10^5$ and $4.91\times10^4 \pm 0.93\times10^5$ respectively. These results came in accordance with those reported by (Elshafey, 2014) who found that the mean values for Saurus ($4.08\ x10^5 \pm 0.71\times10^5$), mackerel ($9.95x10^4 \pm 2.13\times10^4$) and horse mackerel ($3.66x10^4 \pm 0.749\times10^4$). Lower results of total psychrotrophic count(cfu/ g) were obtained by (Shereen et al., 2013) who examined 100 samples of *Tilapia nilotica* collected from different markets in Dakahlia governorate and revealed that the total psychrotrophic count was $3.1\ x10^2 \pm 1.8\ x10^2$ (cfu/g). *Alcaligenes faecalis* was isolated with high incidence followed by *Acinetobacter lwoffii, Acinetobacter baumanii, Acinetobacter calcoaceticus and Flavobacterium aquatile* were the most psychrotrophic bacteria contaminate the examined samples as shown in table (3).

The incidence of Pseudomonas species isolated were 33.3%, 46.7%, 33.3% , 53.3%, 60%, and 40% for fresh tilapia, fresh Mugil cephalus, fresh shrimp, frozen Tilapia, frozen Mugil cephalus, and frozen shrimp, respectively(table 4). Higher results were obtained by (Yagoub, 2009) who examined 150 fish samples and isolated *Pseudomonas* species from 62% of such samples and (Elshafey, 2014) isolate *Pseudomonas* species from frozen Saurus 23(76.67), mackerel 20(66.67) and horse mackerel 19(63.33). While, lower results were reported by (Rahmou- Abeer, 2002) isolated *Pseudomonas* species (28%) from the examined samples and (Abou EL- Atta, 2003) isolated *Pseudomonas* species 26.05%. The most *Pseudomonas* species contaminated these examined samples was *Ps. alcaligenes* and *Ps. fluorescens* followed by *Ps. Putrefaciens* as shown in table (5). The incidence of *Aeromonas* species were 46.7 %and 33.3 %in the examined samples of fresh and frozen Tilapia niloticus, 53.3 % and 40.0% in fresh and frozen *Mugil cephalus*, respectively, 33.3 % and 40.0 %in fresh and frozen shrimp respectively table (6). These results agreed with those reported by (Abou El- Gheit et al., 1995) recorded that the incidence of *Aeromonas* species in Tilapia niloticus was 63.64%. lower results by (Wong et al., 1994 a) isolated *Aeromonas* species from 10% of the examined samples of frozen fish, (Henin, 1995) declared the presence of *Aeromonas* species in incidence in imported frozen fish (15.2%) than fresh water fish (9.7°/o). Fresh cat fish show higher percentages of *Aeromonas organism* (11.6%)

Higher results recorded by (Hafez et al., 1997) that the incidence of motile *Aeromonas septicemia* in Tilapia species was 75%. The most Aeromonas species contaminated these examined samples was *A. hydophila* followed by *A.diversa* followed by *A. punctata* as shown in table (7).

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