Quality Evaluation of Retailed Fresh Water Fish at Alexandria Province

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ABSTRACT:

A total of 150 random samples, of retailed fresh water fish, 50 of each (Tilapia nilotica, Mugil cephalus , Clarius lazera) were randomly collected from different fish markets at Alexandria province are subjected to chemical and microbial analysis for safety and quality assessment .The results showed that the mean values of pH for the examined samples of Tilapia nilotica, Mugil cephalus and lazera were 5.93±0.03, 6.29±0.06 , 6.20±0.04 and 6.39±0.03 mg/100g respectively; the mean values of total volatile nitrogen was 10.62±0.27,8.76±0.31 and 17.51±0.29 mg/100g, respectively; the mean values of trimethylamine were 4.35±0.38, 3.59±0.22 and 4.86±0.15 mg/100g, respectively; the mean values of thiobarbituric acid was 1.18±0.19, 1.82±0.14 and 2.21±0.13 mg/100g, respectively; while, the mean values of free fatty acid were 0.31±0.02, 0.23±0.01and 0.38±0.01 mg/100g, respectively. The results of microbial analysis showed that the mean values of aerobic bacterial count was $8.4\times10^5\pm7.2\times10^4$ for Tilapia nilotica, $4.6\times10^6\pm9.2\times10^5$ for Mugil cephalus and $3.6x10^6\pm8.2x10^5$ cfu/g for Clarius lazera; the mean values of Enterobacteriaceae count were 1.3x105±7.2x104 for Tilapia nilotica, $2.6x10^5\pm9.2x10^4$ for Mugil cephalus and $2.2\times10^5\pm 8.2\times10^4$ cfu/g for Clarius lazera; the mean values of *Staphylococcus aureus* count was $9.2x10^2\pm2.6x10^2$ for Tilapia nilotica, $1.5x10^3\pm7.4x10^2$ for Mugil cephalus and $8.9x10^2\pm5.4x10^2$ cfu/g for Clarius lazera; while, the mean values of mould and yeast count were $3.8x10^2\pm9x10$; $1.9x10^3\pm1.3x10^2$; respectively for Tilapia nilotica, $7.2x10^2\pm2.1x10^2$;$8.9x10^3\pm1.9x10^2$; respectively, for Mugil cephalus and $6.6x10^2\pm1.9x10^2$;$8.9x10^3\pm2.1x10^2$cfu/g for Clarius lazera. This study showed the degree of contamination of Tilapia nilotica, Mugil cephalus and Clarius lazera. In addition, the public health importance of such contaminates has been discussed

Keyword: fresh water fish, pH, Total volatile nitrogen, Trimethylamine, Enterobacteriaceae, Staphylococcus, mould and yeast.

1. INTRODUCTION:

From the nutritional point of view, fish is the best animal protein due to its high nutritive value as well as its high quality. Also, fish is rich in calcium, phosphorous, Iodine, Omega 3, Omega 6 and generous supply of B-complex vitamins as well as its lower content of cholesterol. Due to excessive demand of consumers for fish, imported fish appeared excessively in Egyptian markets so it is necessary to assure its quality to avoid its health hazards so we need to make examination of this fish to know if it is free of spoilage markers although fish flesh, which is the main edible part, is generally sterile immediately after catching, however it may become

contaminated with different micro-organisms during subsequent handling and packaging as these micro-organisms can penetrate from skin and the gut to the flesh (**Brock et al.,1984 and Etzel et al.,1998)** . The penetration and contamination increase in case of fish caught from polluted area where there are high densities of bacteria (**Howgate, 1998)**.

Spoilage markers are very important in judgment of fish quality. pH value was an indication of the extent of microbial spoilage in fish **(Eyo ,2001).** If increased than 6.5 according to E.S. (2005) indicates bad quality of imported fish and needs to total rejection.

Total volatile basic nitrogen analyses reflect only stages of advanced spoilage of fish (**Baixas-Nogueras et al., 2002**)**.** The permissible limit of TVBN which stated by (E.S 2005) is not exceed 30mg/100gm.

Thiobarbituric acid is a widely used as indicator for the assessment of degree of secondary lipid oxidation. An increase in Thiobarbituric acid more than 4.5mg/kg according to E.S. (2005) indicates fat rancidity and needs to total rejection.

Salmonella as a microorganism *(Enterobacteriaceae)* for example affect fish quality for human consumption it acts as a public health hazard if present where it causes food poisoning (**Varnam and Evans, 1991)**.

Enteropathogenic Ecoli may constitute a public health hazard as it may give rise to severe diarrhea in infants and young children as well as food poisoning and gastroenteritis. Among adult consumers (**Banwart, 1989**).

The *Mould* count is used as an index of the proper sanitation and the high-quality products. *Moulds* can assist in the putrefactive process and in other cases; they may impart a mouldy odour and taste to food stuffs. Also, *Mould* can grow over an extremely wide range of temperature, there for one can find *Mould* on particularly all foods at almost any temperature under which foods are held. Besides, *Mould* can assist in the putrefactive process and may produce toxic substances namely mycotoxins which are harmful to human and animals (**Frazier and Westhoff, 1983)**.

Yeast normally plays a small role in spoilage because they constitute only a small portion of the initial population; grow slowly in comparison with most bacteria and their growth may be limited by metabolic substances produced by bacteria. Spoilage *yeasts* find their way into food being widely distributed resulting in undesirable changes in physical appearance (**Walker, 1976).**

In recognition of these public health risk and economic loss of the imported fish this study was undertaken to investigate the following points.

I- Spoilage markers: Determination of pH value, Total volatile nitrogen (TVN), Trimethylamine (TMA), Thiobarbituric acid estimation (TBA) and Free fatty acids.

II- Microbiological evaluation: Aerobic *bacterial* count, *Enterobacteriaceae* count, C*oliforms* count, *Staphylococci count*, *Mould* count and Yeast count**.**

2- MATERIALS AND METHODS

2.1. Sampling:

A total of 150 samples of fresh water fish including, Tilapia nilotica, Mugil cephalus, and Clarias lazera (50 of each) were randomly collected from different markets at Alexandria Province. Samples were kept in a separate sterile plastic bag and transferred in an ice box as soon as to the laboratory of the Food Hygiene Department, Faculty of Veterinary Medicine, Alexandria University under complete aseptic conditions where they were examined for spoilage markers and microbiologically.

2.2. Methods:

2.2.1. Spoilage Index

2.2.1.1. Determination of pH (Pearson, 2006).

2.2.1.2. Determination of Total Volatile Nitrogen (TVN):

Nitrogen (TVN) was recommended by Food and Agriculture Organization **"FAO" (1983).**

2.2.1.3. Determination of Trimethylamine (TMA):

The preparation of samples was carried out as mentioned for TVN using Conway dish according to **FAO (1983).**

2.2.1.4. Determination of Thiobarbituric Acid Number (TBA):

The method adopted for estimation of TBA by **Pikul et al. (1989).**

2.2.1.5. Determination of Free Fatty Acids (FFA):

2.2.1.5.1. Lipid Extraction Using the Folch Method (Folch et al., 1957).

2.2.1.5.2. Titration procedure (Brake and Fennema, 1999).

2.2.2. Microbiological Index

2.2.2.1. Preparation of fish samples for microbiological examinations:

It was performed according to the procedures describe by **ICMSF, (1978).**

2.2.2.2. Total bacterial count (pour plating): was done according to **(Cruickshank et al., 1975).**

2.2.2.3. Isolation and identification of Enterobacteriaceae: was done according to **ISO (2007).**

2.2.2.4. Coliforms count (ICMF, 1998).

2.2.2.5. Staphylococcus count (ICMSF, 1980).

2.2.2.6. Total Yeast and Mould Count: (FAO, 1992).

3- RESULTS and DISCUSSION

3.1. Spoilage markers:

3.1.1. pH:

The measurement of pH value is described as a simple method for detecting fish quality. Where pH is among the most critical factors affecting microbial growth and spoilage of foods in general. The pH value is also a reliable indicator of the degree of freshness or spoilage of fish. The pH value was an indication of the extent of microbial spoilage in fish **(Eyo, 2001**).

Table (1) showed that the pH values of Tilapia nilotica varied from 5.25 to 6.42, with a mean value 6.29±0.06 (not exceed 6.5) **according to Permissible Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).** Also showed that the pH values of Mugil cephalus varied from 6.09 to 6.43 with a mean value 6.20±0.04. Also, the pH values of Cat fish (Clarius lazera) varied from 6.29 to 6.52 with a mean value 6.39±0.03. The above results revealed that pH value in Cat fish (Clarius lazera) is largest than pH of Tilapia nilotica and Mugil cephalus, respectively. The above results may be attributed to poor hygienic measures during, loading, unloading and handling cause increase in pH value and hence rapid spoilage of some samples occurred.

Table (1): Statistical analytical results of pH values in the examined fish samples of retailed fresh water fish (n=50).

Fish species	Min.	Max.	Mean ± S.E*
Tilapia nilotica	5.25	6.42	6.29 ± 0.06
Mugil cephalus	6.09	6.43	6.20 ± 0.04
Clarius lazera	6.29	6.52	6.39 ± 0.03

3.1.2. Total volatile nitrogen (TVB-N):

TVB-N analyses reflect only stages of advanced spoilage of fish, they are considered unreliable for the evaluation of the fish freshness in the early stage of storage and they don't

reflect the mode of spoilage, bacterial or autolytic ***(Huss, 1995; Baixas-Nogueras et al. 2002).***

Table (2&3) showed that the TVB-N (mg/100gm) value of Tilapia nilotica varied from 6.88 to 15.08 with a mean value 10.62±0.27 (mg/100gm) (Not exceed 25mg/100gm) according to according to **Permissible Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).** Also showed that TVB-N (mg/100gm) value of Mugil cephalus varied from 5.03 to 13.57, with a mean 8.76± 0.31 (mg/100gm) and all samples within permissible limit (Not exceed 25mg/100gm). Also showed that TVB-N (mg/100gm) value of Clarius lazera varied from 7.95 to 19.28, with a mean 17.51±0.29 (mg/100gm) and all samples within permissible limit (Not exceed 25mg/100gm). The above results revealed that the TVB-N (mg/100gm) value of Clarius lazera is largest than TVB-N in Tilapia nilotica and Mugil cephlus, respectively. The above results may be attributed to poor hygienic measures during loading, unloading and handling in some markets of Alexandria province which in turn affected the TVB-N value of some samples of Clarius lazera and Tilapia. Although it reflected advanced stage of spoilage in samples above permissible limits in some samples of each Clarius lazera and Tilapia but this not deny early stage of spoilage may be found in other samples which appeared within permissible limits of others so we need farther examination to assure that.

Table (2): Statistical analytical results of Total Volatile Nitrogen "TVN" (mg %) as proteolytic index of spoilage in the examined fish samples of retailed fresh water fish (n=50).

Fish species	Min.	Max.	Mean ± S.E*
Tilapia nilotica	6.88	15.08	10.62 ± 0.27
Mugil cephalus	5.03	13.57	8.76 ± 0.31
Clarius lazera	7.95	19.28	17.15 ± 0.29

Table (3): Acceptability of the examined samples of retailed fresh water fish based on their levels of TVN (n=50).

Fish species	P. L (mg %)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Tilapia nilotica	25	50	100	0	0
Mugil cephlus	25	50	100	0	0
Clarius lazera	25	50	100	0	0

***** Permissible Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).

3.1.3. Trimethyl amine (TMA):

TMA is a widely used as one of freshness assessment indices for proteolytic activity. The data presented in Table (4&5) showed that the TMA (mg/100gm) value of Tilapia nilotica varied from

2.01 to 6.54 with a mean value 4.53±0.38 (mg/100gm) (Not exceed 10mg/100gm) according to according to **Permissible Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).** Also showed that TMA (mg/100gm) value of Mugil cephalus varied from 1.61 to 6.16, with a mean 3.59± 0.22 (mg/100gm) and all samples within permissible limit (Not exceed 10mg/100gm). Also showed that TMA (mg/100gm) value of Clarius lazera varied from 2.17 to 7.96, with a mean 4.86±0.15 (mg/100gm) and all samples within permissible limit (Not exceed 10mg/100gm).

The above results revealed that the TMA (mg/100gm) value of Clarius lazera is largest than TMA in Tilapia nilotica and Mugil cephalus, respectively. The above results may be attributed to poor hygienic measures during loading, unloading and handling in some markets of Alexandria province which in turn affected the TMA value of some samples of Clarius lazera and Tilapia.

Table (4): Statistical analytical results of Trimethyl amine "TMA" (mg%) as proteolytic index of spoilage in the examined samples of retailed fresh water fish (n=50).

Fish species	Min.	Max.	Mean ± S.E*
Tilapia nilotica	2.01	6.59	4.35 ± 0.38
Mugil cephalus	1.61	6.16	3.59 ± 0.22
Clarius lazera	2.17	7.96	4.86 ± 0.15

Table (5): Acceptability of the examined samples of retailed fresh water fish based on their levels of TMA (n=50).

Fish species	P. L (mg %)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Tilapia nilotica	10	30	100	0	0
Mugil cephalus	10	30	100	0	0
Clarius lazera	10	30	100	0	0

***** Permissible Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).

3.1.4. Thiobarbituric acid (TBA):

TBA is a widely used as indicator for the assessment of degree of secondary lipid oxidation ***(Gulsun Ozyurt et al, 2008)***.

Table (6&7) showed that the TBA (mg/kg flesh of fish) value of Tilapia nilotica varied from 0.78 to 2.90 with a mean value 1.18±0.19 mg/kg flesh and all samples within permissible limits (not exceed 4.5 mg/kg) according to according to **Permissible Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).** Also showed that the TBA (mg/kg flesh of fish) value of Mugil cephalus varied from 1.18 to 3.05 with a mean value 1.82±0.14 mg/kg flesh and all samples within permissible limits (not exceed 4.5 mg/kg).

Table (6): Statistical analytical results of Thiobarbituric acid "TBA" (mg%) as lipolytic index of spoilage in the examined samples of retailed fresh water fish (n=50).

Fish species	Min.	Max.	Mean ± S.E*
Tilapia nilotica	0.78	2.90	1.18 ± 0.19
Mugil cephalus	1.18	3.05	1.82 ± 0.14
Clarius lazera	1.28	3.25	2.21 ± 0.13

Also showed that the TBA (mg/kg flesh of fish) value of Clarius lazera varied from 1.28 to 3.25 with a mean value 2.21±0.13 mg/kg flesh and all samples within permissible limits (not exceed 4.5 mg/kg). The above results revealed that the TBA value in Clarius lazera was largest than the TBA in Mugil cephalus and, Tilapia nilotica, respectively. The above results may be attributed to poor hygienic measures during loading, unloading and handling in some markets of Alexandria.

Table (7): Acceptability of the examined samples of retailed fresh water fish based on their levels of TBA (n=50).

Fish species	P. L (mg/Kg) *	Accepted samples		Unaccepted samples	
		No.	%	No.	
Tilapia nilotica	4.5	50	100	0	0
Mugil cephalus	4.5	50	100	0	0
Clarius lazera	4.5	50	100	0	0

***** Permissible Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).

3.1.5. Free fatty acids (FFA):

The results were given in Table (8) revealed that that the free fatty acids (FFA) in examined different species of fish varied from 0.18 to 0.42with a mean value for 0.31±0.02 Tilapia; 0.14 to 0.35 mg% with an average 0.14±0.35mg% for Mugil cephalus and 0.28 to 0.49 mg% with an average 0.38±0.01mg% for Clarius lazera, respectively. The difference between the experimental samples of different fish species was non-significant as shown in table (8). The results were lower than those obtained by (**Gülsün et al., 2009; Nazemroaya et al., 2011 and Vafakhah et al.,2014).**

Table (8): Statistical analytical results of free fatty acids (mg %) as lipolytic index of spoilage in the examined samples of retailed fresh water fish (n=50).

Fish species	Min.	Max.	Mean ± S.E*
Tilapia nilotica	0.18	0.42	0.31 ± 0.02
Mugil cephlus	0.14	0.35	0.23 ± 0.01
Clarius lazera	0.28	0.49	0.38 ± 0.01

Free fatty acids formation during ice storage (hydrolytic rancidity) is due to the presence of Poly un saturated FA leading to negative sensory impact related to FFAs **(Bremner,2002)**.

They have shown to undergo faster oxidation rate than bigger lipid classes of triglycerides and phospholipids which affect the dietary quality of fish **(Losada et al.,2007).** The susceptibility to rancidity depend not only on the amount of lipid present, but also the lipid composition **(Nazemroaya et al., 2011)**. Fat spoilage consider as the most important factor that lower fish quality **(Connell ,2002)** as it produces undesirable taste **(Balachandran,2001)**, color and nutritional value of fish **(Clark et al. 1997).** FFA changes were recorded as indicator of hydrolytic rancidity **(Vafakhah et al., 2014).**

3.2. Microbiological evaluation:

We need to microbiological examinations of fish samples to evaluate the possible presence of bacteria or organisms of public health significance and to give an impression of the hygienic quality.

3.2.1. Aerobic plate count:

The Aerobic *plate* count (APC) (cfu /g) is intended to indicate the level of microorganism in product (**Food and Drug Administration, 2001**).

Table (9), showed that the *Aerobic bacterial* count (cfu/g) of the different examined fish samples varied from $3.1x10^4$ to $1.4x10^6$ with a mean value of $8.4x10^5 \pm 7.2x10^4$ for Tilapia nilotica with a number of samples above permissible limits were 21 (42%) (Not exceed $10^{6)}$ **according to Egyptian Organization for Standardization "EOS" (2005).**

Table (9): Statistical analytical results of Aerobic Plate Count (CFU/g) of examined samples of retailed fresh water fishes at Alexandria provinces (n =50/ each)

Fish species	Min	Max	Mean	±SEM	Samples exceeding the permissible limit	
					No	%
Tilapia nilotica	3.1×10^4	1.4×10^6	8.4×10^{5b}	7.2×10^4	21	42
Mugil cephelus	4.2×10^4	3.1×10^7	4.6×10^{6a}	9.2×10^5	23	46
Clarius lazera	2.3×10^4	1.3×10^7	3.6×10^{6a}	8.2×10^5	22	44

Means with similar letters are not significantly different at $P \leq 0.05$.

Aerobic plat count must not exceed 10^5 CFU/g according to ES, (2005).Also, data showed that the *Aerobic bacterial* count of Mugil cephalus varied from $4.2x10^4$ to $3.1x10^7$ with a mean value of $4.6x10^6 \pm 9.2x10^5$ with a number of samples above permissible limits were 23 (46%). Also, data revealed that the *Aerobic bacterial* count of Clarius lazera varied from $2.3x10^4$ to $1.3x10^7$ with a mean value of $3.6x10^6 \pm 8.24x10^5$ with a number of samples above permissible limits were 22 (44%). These variations could be attributed to the fish species, environments, methods of catch, extend of handling during transportation and distribution as well as marketing **(Wang et al., 1994).**

3.2.2. *Enterobacteriaceae* count:

Table (10), revealed that the *Enterobacteriaceae* count (cfu/g) of Tilapia nilotica varied from $2.4x10^4$ to $4.0x10^6$ with a mean value of $2.1x10^5 \pm 0.81x10^5$(cfu/g) in 50 (100%) of examined samples. Also showed that the *Enterobacteriaceae* count (cfu/g) of Mugil cephalus varied from $1.9x10^4$ to $4.5x10^6$ with a mean value of $6.7x10^5 \pm 1.7x10^5$ (cfu/g) in 50(100%) of examined samples. Also showed that the *Enterobacteriaceae* count (cfu/g) of Clarius lazera varied from $3.0x10^4$to $3.9x10^6$ with a mean value of $6.8x10^5 \pm 1.4x10^5$ in 50(100%) of examined samples. Mugil cephalus & Clarius lazera had largest mean value of *Enterobacteriaceae* count than other species.

The mentioned results may be attributed to poor hygienic measures in some markets of Alexandria which in turn affected. Enterobacteriaceae in fish are considered as an indicator to sewage pollution and has been reported as opportunistic pathogen in fish **(Rajasekaran, 2008).** The pathogenic strains of Enterobacteriaceae may cause diarrhea in fish **(Shender et al., 2009).**

Table (10): Statistical analytical results of *Enterobacteriaceae* count (CFU/g) of examined samples of retailed fresh water fishes at Alexandria provinces (n =50/ each)

Fish species	Min	Max	Mean	±SEM
Tilapia nilotica	2.4×10^4	3.2×10^5	1.3×10^{5b}	7.2×10^4
Mugil cephelus	3.1×10^4	4.3×10^5	2.6×10^{5a}	9.2×10^4
Clarius lazera	2.9×10^4	4.1×10^5	2.2×10^{5a}	8.2×10^4

Means with similar letters are not significantly different at $P \leq 0.05$. There is no permissible limit for Enterobacteriaceae recommended by ES, (2005).

3.2.3. Staphylococci count cfu/g):

Data presented in Table (11), revealed that the *Staphylococcus aureus* count (cfu/g) of Tilapia nilotica varied from $1.9x10^2$ to $4.2x10^3$ with a mean value of $9.2x10^2 \pm 2.6x10^2$ (cfu/g) in 50 (100%) of examined samples. Also showed that the *Staphylococcus aureus* count (cfu/g) of Mugil cephalus varied from $2.8x10^2$ to $5.2x10^3$ with a mean value of $1.5x10^3 \pm 7.4x10^2$ (cfu/g) in 50 (100%) of examined samples.

While, the *Staphylococcus aureus* count (cfu/g) of Clarius lazera varied from $1.2x10^2$ to $4.1x10^3$ with a mean value of $8.9x10^2 \pm 5.4x10^2$ in 50 (100%) of examined samples. Mugil cephalus had largest mean value of *Staphylococcus aureus* count than other species. The above results may be attributed to poor hygienic measures in some markets of Alexandria which in turn affected. Also, may be due to the fish species, environments, methods of catch, extend of handling during transportation and distribution as well as marketing **(Wang et al., 1994).**

Table (11): Statistical analytical results of Staphylococci Count (CFU/g) of examined samples of retailed Fresh water fishes at Alexandria province (n =50/ each)

Fish species	Min	Max	Mean	±SEM
Tilapia nilotica	1.9×10^{2}	4.2×10^{3}	9.2×10^{2a}	2.6×10^{2}
Mugil cephlus	2.8×10^{2}	5.2×10^{3}	1.5×10^{3b}	7.4×10^{2}
Clarius lazera	1.2×10^{2}	4.1×10^{3}	8.9×10^{2a}	5.4×10^{2}

Means with similar letters are not significantly different at P ≤0.05. Fresh water fish must be free from Staphylococci according to ES, (2005). All of the examined samples of fish carcasses had staphylococcal count exceeding the permissible limit.

4.2.4. Mould count (cfu/g):

Mould can grow in extremely wide range of temperature. Therefore, we can find *mould* particularly in all food at any temperature under which food is held, on other hand *mould* can help in putrefaction and some species can produce mycotoxins which are harmful for human and animals **(Frazier and Westhoff,1983).**

Table (12), revealed that the *Mould* counts (cfu/g) of Tilapia nilotica varied from 1.0x10 to $1.3x10^{3}$ with a mean value of $3.8x10^{2}\pm9x10$ (cfu/g) in all examined samples 50 (100%). While, in Mugil cephalus varied from 8.0x10 to $4.9x10^{3}$ with a mean value of $7.2x10^{2}\pm2.1x10^{2}$ in all examined samples. Also, in Clarius lazera varied from 7.0x10 to $4.71x10^{3}$ with a mean value of $6.6x10^{2}\pm1.9x10^{2}$ in all examined samples. These variations could be attributed to the fish species, environments, methods of catch, extend of handling during transportation and distribution as well as marketing **(Wang et al., 1994).** These results reveal bad quality of fish samples in these markets.

Table (12): Statistical analytical results of Molds Count (CFU/g) of examined retailed fresh water fishes at Alexandria province (n =50/ each)

Fish species	Min	Max	Mean	±SEM
Tilapia nilotica	1×10	1.3×10^{3}	3.8×10^{2b}	9×10
Mugil cephlus	8×10	4.9×10^{3}	7.2×10^{2a}	2.1×10^{2}
Clarius lazera	7×10	4.7×10^{3}	6.6×10^{2a}	1.9×10^{2}

Means with similar letters are not significantly different at P ≤0.05. Fresh water fish must be free from molds according to ES, (2005). All of the examined samples of fresh water fish had molds count exceeding the permissible limit.

4.2.5. Yeast count (cfu/g):

The presence of *Yeast* indicates poor hygienic measures and high-water contamination and some species constitutes a public health hazard, as it may cause gastrointestinal disturbances, valvovaginits, endocarditis, pulmonary infection and occasionally fatal systemic disease **(Jesenska and Hardinova, 1981)**.

Table (13), showed that the *Yeast* count of Tilapia nilotica varied from 5.0x10 to $5.3x10^3$ with a mean value of $1.9x10^3 \pm 1.3x10^2$ in all examined samples (100%). Also in Mugil cephalus varied from $1.6x10^2$ to $3.1x10^4$ with a mean value of $8.9x10^3 \pm 1.9x10^2$ cfu/g in all examined samples (100%). While, in Clarius lazera varied from $1.4x10^2$ to $3.1x10^4$ with a mean value of $8.9x10^3 \pm 2.1x10^2$ in all examined samples (100%). These variations could be attributed to the fish species, environments, methods of catch, extend of handling during transportation and distribution as well as marketing **(Wang et al., 1994).** These results reveal bad quality of fish samples in these markets.

Table (13): Statistical analytical results of Yeasts Count (CFU/g) of examined samples of retailed fresh water fishes at Alexandria province (n =50/ each)

Fish species	Min	Max	Mean	±SEM
Tilapia nilotica	5×10	5.3×10^3	1.9×10^{3b}	1.3×10^2
Mugil cephlus	1.6×10^2	3.1×10^4	8.9×10^{3a}	1.9×10^2
Clarius lazera	1.4×10^2	3.1×10^4	8.9×10^{3a}	2.1×10^2

Means with similar letters are not significantly different at P ≤0.05. Fresh water fish must be free from yeasts according to ES, (2005). All of the examined samples of fresh water fish had yeasts count exceeding the permissible limit**.**

4-CONCLUSION

The importance of using measures focused on the hygienic quality of both raw material and processing units to avoid high bacterial load of fish, the raw fish. Fish samples must be of very low initial bacterial count, application of the HACCP system during handling of these samples, educational programs must be applied to the workers as learning of such workers about sources of contamination of such samples and personal hygiene such as, cleaning of their hands after toilet and wearing muzzles on mouth and nose. Also, cleaning and sanitation of machines used for handling after each lot to avoid cross contamination.

5-REFERENCES

Baixas-Nogueras S., Bover-Cid S., Vidal-Carou M.C., Veciana-Nogués M.T., Mariné-Font A. 2002. Trimethylamine and total volatile basic nitrogen determination by flow injection/gas diffusion in Mediterranean hake (Merluccius merluccius). Journal of Agricultural and Food Chemistry, 49 (4): 1681-1686.

Balachandran, KK. 2001. On board handling and preservation in postharvest technology of fish and fish product. India, 440 p.

Banwart, G. 1989. Basic food microbiological 2nd Ed. An. Avi. Book, Published by Van Nostrand Reinhold, New York. Ed. Appendix 5, pp.258.

Brake, N. C., Fennema, O. R. 1999. Lipolysis and lipid oxidation in frozen minced mackerel as related to molecular diffusion and presence of gelatin. Biochem., 61: 125- 132.

Bremner, H.A. 2002. Safety and quality issues in fish processing. Wood head Publishing Limited, Food Science and Technology, Cambridge, 519 PP.

Brock, M. J. M., Mossel, D. A. A., Mol, H. 1984. Microbiological quality of retail fresh fish fillets in Netherlands. International J. Food. Microbiol., 1: 53-61.

Clark, E.M., Mahoney, A.W., Carpenter, L.E. 1997. Heme and total Iron in ready-to-eat Chicken. Journal of Agricultural and Food Chemistry 45:124-126.

Connell, J.J. 2002. Quality control in fish Industry. Torry Advisory Note NO.58.

Cruickshank, R., Duguid, J. P., Marmion, B. P., Swain, R. H. A. 1975. Medical Microbiology, 12th Ed., Churchill Livingstone Edinburgh, London and New York.

Egyptian Organization for Standardization "EOS" 2005. Reports related to No. 3494/2005 for chilled fish. Egyptian Standards, Ministry of Industry, Egypt.

Etzel. V., Meyer, C., Ballin, U., Krause, R. 1998. Investigation for the evaluation of quality of Nile Prech after arriving at German Wholesars. FSTA, 30 (12): 226.

Eyo A.A. 2001. Fish processing technology in the tropics. University of Illorin press, Nigrria, 23-34.

Eyo A.A. 2001. Fish processing technology in the tropics.University of Illorin press, Nigrria, pp. 23-34.

FAO, 1983. Support and Development of the Retail Trade in Perishable Fishery Products. Pp 10-13 (Eddie, G.C. Edit.) FAO fisheries Technical Paper No. 235 FIIU / T235.

FAO, 1992. Manual of food quality control. 4 Rev. 1. Microbiological analysis (Andrews, W. ed.) FAO food and nutrition paper NO.14/ 4 Rev. 1.

FDA (Food and Drug Administration) 2001. Department of Health and Human services.FDA&EPA safety levels in Regulations and Guidance. In Fish and fisheries products Hazards and controls Guidance: Third Ed. Appendix 5, pp.258.

Folch, J., Lees, M., Sloanes, G. 1957. Simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497- 509.

Food and Agriculture Organization "FAO" 1980. Manual of Food Quality Control. FAO, United Nation, Rome, Italy. Seafood, Washington, DC.

Frazier, W.C. Westhoff, D.C. 1983. Food microbiological. 2nd Ed., Tata Mc-Grow-Hall Publishing Company limited New-Delhi.

Frazier, W.C., Westhoff, D.C. 1983. Food microbiological. 2nd Ed., Tata Mc-Grow-Hall Publishing Company limited New-Delhi.

Gulsun Ozyurt, Esmeray Kuley, Serhat Ozkutuk, Fatih, zogul 2008. Sensory, microbiological and chemical assessment of the freshness of red mullet (Mullus barbatus) and goldband goatfish (Upeneus moluccensis) during storage in ice. In Food Chemistry 114 :505–510**.**

Gülsün, O., Esmeray,K., Serhat,O., Fatih O. 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet (Mullus barbatus) and goldband goatfish (Upeneus moluccensis) during storage in ice. Food Chemistry 114: 505–510.

Howgate, P. 1998. Review of the public health safety of products from aquaculture. International J. Food Sci. Techno. 33: 99-125.

Huss H.H. 1995. Quality and quality changes in fresh fish. FAO Fisheries Technical Paper 348, FAO.

Huss, H. H., Ababouch, L., Gram L. 2003. Assessment and management of seafood safety and quality. FAO Fisheries Technical Paper. No. 444. Rome: FAO.

ICMSF (International Commission of Microbiological Specification for Foods) 1978. Microbial ecology of foods, vol.2, Food commodities. Academic Press, New York, PP.580-581.

ICMSF (International Commission of Microbiological Specification for Foods) 1980. Microbial ecology of foods, Vol. 2, Food Commodities. Academic Pres, New York, PP. 580 - 581.

ICMSF (International Commission of Microbiological Specification for Foods) 1998. Micro-organisms in foods. Microbial Ecology of food commodities. Blackie Academic and Professional, London, New York, Tokyo, Melbourne. Madress.

Iso 2007. Microbiology of food and animal feeding stuffs-General requirement and guidance for microbiological examination. third ED.ISO.org. published in Switzerland.

Jesenska, Z., Hrdinova, I. 1981. Moulds in food in Czechoslovakia Zeitschrift Fur Lebensm, Unters Forsch., 173 (1): 16-20.

Losada, V., Barros-Velazques, J., Aubourg, S. P. 2007. Rancidity development in frozen Fish: Influence of slurry Ice as preliminary chilling treatment. LWT, 40: 991-999.

Nazemroaya, S., Sahari, M.A., Rezaei, M. 2011. Identification of fatty acid in Mackerel and shark fillets and their change during six months of frozen storage at -18. J. Agr. Sci. tech., 13: 553:566.

Pearson, D. 2006. Chemical Analysis of Foods. 11th Ed, Publishing Co., Churchill Livingstone, Edinburgh, London, United Kingdom.

Pikul, J., Leszezynski, D.E., Kummerow, F. 1989. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. J. Agri. Food Chem., 37:1309.

Rajasekaran, p. 2008. Enterobacteriaceae group of organisms in sewage –fed fishes. Advanced Biotech. 8 :12-14.

Shender, L.A., Spraker, T.R. 2009. Salmonellosis in a free-ranging population of javelinas (Pecari tajacu) in south central Arizon. J. Wild Dis., 45(4): 941-951.

Vafakhah, F. O. H. and Javaheri B. M. (2014): Lipid quality in benni (Barbus sharpeyi) fillets during ice storage. International Journal of Biosciences 4 (6): 109-116.

Varnam, A. H. Evans, M.G. 1991. Food borne pathogens. Wolfe Publishing Ltd. Aylesbury, England.

Walker, H.W. 1976. Spoilage of food by Yeast. Food Technil. 57:61.

Wang, S.J., Chen, J.M., Fan, J.J. 1994. Quality changes in fresh Tilapia and Milkfish during refrigerated (4-degree C) and frozen (-15-degree C) storage J. Food Analysis, 2(4):311 – 316.