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Assessment of the Bacterial Quality and Toxic Heavy Metal Residues of Frozen Fish Fillet In Kaferelsheikh Markets

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ABSTRACT

Fish has high consumer preference due to its inherent nutritive value, taste and easy digestibility. It is one of the most important sources of animal protein available and has widely been accepted as a good source of animal protein and other elements for maintenance of healthy body. The present study was planned to estimate the quality and heavy metal residues of the imported frozen fish fillet for that aim, a total of 50 imported frozen fish fillet samples from market retail from different origins in Kafr El-Sheikh Governorate. Bacteriological and Chemical analysis was done for imported frozen fish fillet. The results revealed that total bacterial count, coliform count and total psychtrophic count were 32.04x107 ± 5.75x107, 14.35x103±5.26x103 and 8.08x108±13.46x108 respectively. The *Staphylococcus aureus* were isolated from 23 samples (46%) detection of Staph. aureus enterotoxin by using multiplex PCR indicates that only 9 (39.1%) strains from the 23 isolates were positive for enterotoxin production, 6 (26%) isolates produce Sea; 2(8.7%) isolates produce Seb; one (4.3%) isolate Sec; and one (4.3%) isolate produce Sed enterotoxin. On the other hand, E.coli was detected in 5 samples (10%) of the examined sample, and the serological identification of the obtained isolates revealed the presence of the following serotypes 1(0.2%) for EPEC (O153:H2), 2(0.4%) for ETEC (O126:H21) and 1(0.2%) for EHEC (O55:H7), 1(0.2%) for EHEC (O121:H7). While in heavy metal residues were found that mean values of Mercury, Lead and Cadmium were 0.5992±0.102, 0.1596±0.037 and 0.059±0.019 mg/kg respectively. The obtained results indicate that consumption of imported frozen fish fillet may cause a public health hazard to the consumer. Measures to control the quality of imported frozen fish fillet during handling and processing should be taken.

1. INTRODUCTİON

Fishery products are important not only from a nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world (Abisoye, *et al.*, 2011).

Safety of the fish products and their quality assurance is one of the main problems of food industry today. The presence or absence of food borne pathogens in a fish product is a function of harvest environment, sanitary conditions, and practice associated with equipment and persons in the process environment (FAD, 2001 and Huss, 2003).

Consuming fish provides an important source of protein, polyunsaturated fatty acids (PUFA), liposoluble vitamins and essential minerals, which are associated with health benefits and normal growth (Davigluset *al.*, 2002; Verbekeet *al.*, 2007). According to FAO statistics, fish accounted for about 16% of the global population's intake of animal protein and 6% of all protein consumed (FAO, 2010).

Some of microbiological methods used to assess fish freshness are total plate count, total coliform and fecal coliform. Total plate count is good indicator of the sensory quality or expected shelf life of the product (Olafsdottiret *al.*, 2006; Koutsoumanis and Nychas, 2000).

Eklundet *al.*, (2004) recorded that contamination of fish by pathogens particularly staphyloccousaureus and Escherichia coli

O^{157}:H^{7}may occur prior to harvest, during capture, processing, distribution and/or storage.

Many chemical elements that are present in seafood are essential for human life at low concentrations, but can be toxic at high concentrations. Other elements such as mercury (Hg), cadmium (Cd) and lead (Pb) have no known essential function in life and are toxic even at low concentrations when ingested over a long period. Therefore many consumers regard any presence of these elements in fish as a hazard to health (Oehlenschlger, 2005).

Trace metals are generally released in aquatic environments in different ways and accumulation of these metals is dependent on the concentration of the metal and the exposure period. Levels of heavy metals in fish have been widely reported (Romeo *et al.*, 1999, Edwards *et al.*, 2001, Gaspic*et al.*, 2002, Sataruge*t al.*, 2003, Küçüksezgin*et al.*, 2006). Cadmium has not been found to occur naturally in its pure state and its concentration seems to be directly proportional to zinc (Zn) and lead (Pb) concentrations. Therefore, the aim of this study was to evaluate the quality of frozen fish fillet in kaferElsheikh markets through bacteriological testes and to determine the levels of heavy metals like mercury (Hg), cadmium (Cd) and lead (Pb) in the muscles of frozen fish fillet.

Humans can be exposed to toxic heavy metals through various sources. Water pipes, paint and gasoline are common sources of Pb exposure. At low concentrations Pb can affect learning and memory and at higher doses it can cause poisoning . Humans are usually exposed to methyl mercury (Hg) through consumption of fish such as shark, swordfish and tuna. There is also bioaccumulation of Hg in fresh water fishes that live in contaminated lakes. Consumption of contaminated fish can pass the mercury to humans. Mercury at high doses can cause cerebral-palsy in children and cadmium can affect the male reproductive systems. Dental amalgam filling is one of the main routes for inorganic mercury contamination. Cadmium (Cd) exposure can come from semi-conductor manufacture, metal plating, ceramic plating, shellfish and contaminated water (NRDC,2005).

1. MATERİAL AND METHODS

1.1.Collection of samples:

Fifty frozen fish fillet samples were randomly collected from kafer El-sheikh markets.Each sample was weighted 100g packed in polyethylene bags and put in an insulated ice-box filled with crushed ice, then immediately transferred without delay to Food Hygiene Laboratory for bacteriological and chemical evaluations.

1.2.**Preparation of the Samples** (ISO/IEC, 1999)**.**

Each closed (sealed) sample package was thawed by holding it in refrigerator overnight at 5°c then , Accurately 10g from each sample was aseptically cut and transferred into a sterile polythene stomacher bag and blended with 90ml sterile normal saline (Stomacher 400, Seaward medicals, UK.) at 230 rpm for 60 seconds. Then, one ml of the homogenate was aseptically transferred into 9 ml normal saline in test tube. Similarly, further dilutions required for inoculation was prepared by this decimal serial dilution process.

A- Bacteriological tests:

1-**Total bacterial count** was enumerated in standard plate count agar after incubation at 37 ^{0}C for 48 h as described by (USDA, 1998).

2- Total coliform count were enumerated in lauryl sulphate broth (LST) after incubation at 35 ^{0}C for 48 h to evaluate gas formation then transferred to Brilliant Green Lactose Bile broth (BGLB) incubated at 35 ^{0}C for 48 h according to (AOAC,1980).

2.3. Total Psychtrophic count was enumerated in standard plate count agar after incubation at a 7°C for 10 days as described by (Swanson *et al.*, 1992).

2.4. Isolation and identification of *Staph. aureus*:Was carried out using slandered methods of (USFDA- BAM,2001) selective medium used for *Staph. aureus* was Baird-parker agar inoculated plates were incubated at 37 ^{0}C for 48 h. Characteristic appearance of black shining convex colonies of 1:1.5 mm in diameter with narrow white margin and surrounded by a clear area extending into opaque medium were considered to be presumptive Staph. aureus. The obtained colonies were picked up and stored in semisolid agar for further identification, morphologically, microscopically and biochemically according to (MacFaddin, 2000).

Identification of some enterotoxin genes of *S. aureus* by using Multiplex Polymerase Chain Reaction (PCR):

2.4.1. Primer sequences used for PCR identification system:

Application of PCR for identification of enterotoxin genes (A, B, C & D) of *S. aureus* was performed essentially by using Primers (Pharmacia Biotech) as shown in the following table 1.

2. DNA Extraction using QIA amp kit according to (Shah **et al.,** 2009).

3. Amplification of enterotoxin genes of *S. aureus* according to (Mehrotra et al., 2000).

5- E. coli: Eosin-methylene blue agar (EMB), were used, which was selective and differential for fecal Coliforms (Escherichia coli), after incubation at 37^0C for 24 hours, the plates were examined for typical Escherichia coli colonies, which are metallic green sheen. A number (2-3) of isolated colonies were picked out and purified on EMB, then subcultured to Nutrient agar slants and incubated at 35^0C for 18 -24 hours to be tested completely through the complete test cited by (APHA,1992).

Serodiagnosis of *E. coli*:

The isolates were serologically identified according to Kok et al. (1996) by using rapid diagnostic E. coli antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

B- Determination of heavy metals:

1. Washing procedure (Järup, 2003):

The test tubes, polyethylene tubes and glassware were soaked in water and soap for 2 hours then rinsed several times with tap water. Moreover, the glassware was rinsed once with distilled water, once with cleaning mixture (520 ml deionized water, 200 ml concentrated HCl and 80 ml H_2O_2) and once with washing acid (10% HNO_3). Finally, they were washed with deionized water and then air-dried in incubator away from contamination or dust.

2. Digestion procedure according to (Staniskiene*et al.,* 2006).

3. Preparation of blank and standard solution according to (Andreji*et al.,* 2005).

4. Determination:

Instrumental procedures for various analyses of heavy metals were based on those suggested in the operator manual of the flame Atomic Absorption Spectrophotometer (UNICAM969AA Spectronic, USA). Accurately, the apparatus was adjusted at wave lengths of 253.7 for mercury, 217.0 nm for lead and 228.8 nm for cadmium. Absorbance and concentration of each metal were recorded on the digital scale of the apparatus. The obtained results of mercury, lead and cadmium levels in the examined samples were calculated as mg/kg on wet weight.

Table 1. Primer sequences used in this study

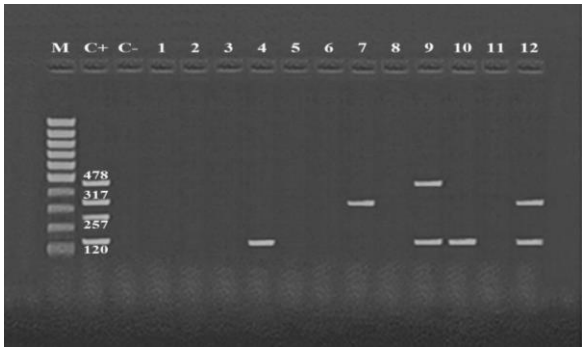
Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
sea (F)	**5′** TTGGAAACGGTTAAAACGAA**′3**	120	Rall*et al.,* (2008)
sea (R)	**5′** GAACCTTCCCATCAAAAACA **′3**		
seb (F)	**5′** TCGCATCAAACTGACAAACG **′3**	478	
seb (R)	**5′** GCGGTACTCTATAAGTGCC **′3**		
sec (F)	**5′** GACATAAAAGCTAGGAATTT **′3**	257	
sec (R)	**5′** AAATCGGATTAACATTATCC **′3**		
sed (F)	**5′** CTAGTTTGGTAATATCTCCT **′3**	317	
sed (R)	**5′** TAATGCTATATCTTATAGGG **′3**		

2. RESULTS AND DISCUSSİON

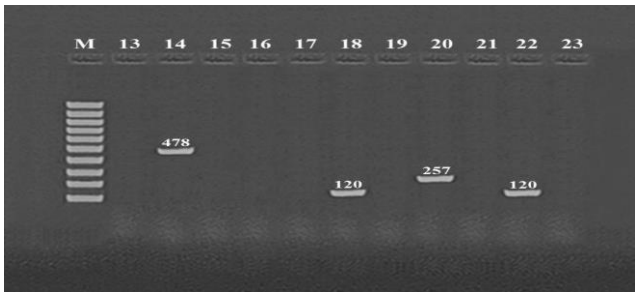
Table (2):Bacterial count of fish fillet samples (n=50) (CFU/ g fish):

	No. of examined samples	No. of positive samples		bacterial count (CFU/g)		
		No.	*%*	*Max.*	*Min.*	*Mean ±SE.*
Total bacterial count	50	48	96	$2x10^9$	1×10^7	$32.04x10^7\pm5.75\ x10^7$
Coli form count	50	34	68	$1.5x10^5$	$1\ x10^3$	$14.35x10^3\pm5.26\ x10^3$
***Psychtrophic* count**	50	46	92	2.710^9	1×10^7	$8.08x10^8\pm3.46\ x10^8$

Value is presented as maximum, minimum, mean ±standard errors.



Photograph (1): Agarose gel electrophoresis of multiplex PCR of sea (120 bp), seb (478 bp), sec (257 bp), and sed (317 bp) enterotoxin genes for characterization of *S. aureus*. Lane M: 100 bpladder as molecular size DNA marker.Lane C+: Control positive for for sea, seb, sec, sed and see genes. Lane C-: Control negative.Lanes 4 & 10: Positive *S. aureus* strains for sea gene.Lane 7: Positive *S. aureus* strain for sed gene. Lane 9: Positive *S. aureus* strain for sea and seb genes. Lane 12: Positive *S. aureus* strain for sea and sed genes.Lanes 1, 2, 3, 5, 6, 8 & 11: Negative *S.aureus* strains for enterotoxins.



Photograph (2): Agarose gel electrophoresis of multiplex PCR of sea. (120 bp), seb (478 bp), sec (257 bp), and sed (317 bp) enterotoxin genes for characterization of S. aureus.Lane M: 100 bp ladder as molecular size DNA marker. Lanes 18 & 22: Positive S. aureus strains for sea gene. Lane 14: Positive S. aureus strain for seb gene. Lane 20: Positive S. aureus strain for sec gene. Lanes 13, 15, 16, 17, 19, 21 & 23: Negative S.aureus strains for enterotoxins.

Table (3): Incidence of *Staph. aureus* in examined fish fillet samples:

No. Of samples	+Ve samples	%	-Ve samples	%
50	23	46	27	54

Table (4): Incidence of E.coli in examined fish fillet samples:

No. Of samples	+Ve samples	%	-Ve samples	%
50	5	10	45	90

Table (5): Serological identification of *E. coli*:

Strain characterization	Serodiagnosis	No. of strain	%
EPEC	**O153 : H2**	**1**	**20**
ETEC	**O126 : H21**	**2**	**40**
EHEC	**O55 : H7**	**1**	**20**
EHEC	**O121 : H7**	**1**	**20**

Table (6): Determination of Heavy metals (mg/Kg) "ppm" in fish fillet sample.

Heavy metal examined	*No.*	*Max.*	*Min.*	*Mean± SE*	*Permissible Limit* (mg/Kg)	*Max.daily intake(µg/kg b.w/day)*
Mercury	50	2.58	0	0.5992±0.102	0.50	0.09
Lead	50	0.9	0	0.1596±0.037	0.10	0.40
Cadmium	50	0.65	0	0.059± 0.019	0.10	0.24

Values were presented as maximum, minimum, mean ±standard errors.
Permissible limit according to EOS (2005).

The microbiological quality of frozen fish fillet depended upon various factors such as the nature of the raw material, its pre and post-harvest treatments and the sanitary condition of the processing factories, the rate and nature of freezing, the temperature and length of storage, the original numbers, growth during storage, thawing process and physical protection offered by the food.

As been shown in Table (2), the mean values for total bacterial count were $32.04x10^7 \pm 5.75\ x10^7$ CFU/gm fish meat. Our result agreed with Yeasmin et al. (2010) who reported an increase of bacterial load from 7.5×10^3 to 6.2×10^9 CFU/g and higher than (Gnanambal and Patterson,2005) ,(Mohamed et al.,2009) and (Sanjee and Karim,2016) that reported total bacterial count of frozen fish fillet from 10^3 to $l0^6$ cfu/g , $9.7\times10^5 \pm 2.05\times10^6$cfu/g and 2.8×10^5 to 4.9×10^5cfu/g respectively. Total bacterial count could reflect the quality of food sanitation during manufacturing, shipping and storage, and also provides an index of food freshness (Jyh-Wei and Yin-Hung,2000) and the bacterial load in frozen products is an indication of the initial bacterial load in the product before freezing, hygiene of processing plant, effectiveness of freezing, post process handling and time temperature abuse during storage and transportation.

Total Coliform count in our study was $14.35x10^3 \pm 5.26x10^3$,nearly similar result was obtained by (Mohamed et al.,2009) that reported total Coliform count of frozen fish fillet were $2.2\times10^2 \pm 3.9\times10^2$cfu/g, lower result was obtained by (Jay *et al.*, 2005) 2.20-2.32 log MPN/g. The presence of total Coliform count is indicator of sewage contamination which may also occur during different processing steps such as transport and handling .Moreover, the contamination may also be caused by the water used for washing or icing (Boyd ,1990). The high incidence of total coliforms at the end of storage day corroborates the findings of (Arannilewa et al. 2005) which observed an increasing of total coliforms count with a prolonged storage of fish fillet. The increased total coliforms may be attributed to fillet quality, temperature fluctuations, time taken during the processing and time taken to transport fish (Mhango*et al.*, 2010).

Total Psychrotrophic count in our study were $8.08x10^8 \pm 3.46\ x10^8$ CFU/gm fish, these results were nearly similar to (Gram et al., 1989) who mentioned that Total Psychrotrophic count were (10^9) cfu/gram fish meat and higher than (Nancy et al.,2013) who found that Total Psychrotrophic count were$2.4\ x\ 10^5 \pm 6\ x\ 10^4$ CFU/gm fish fillet. The importance for recording the psychrotrophic count is due to that, they may be related to level of bacterial contamination of fish which depended on the environment source which include mesophiles that can tolerate and grow in low temperatures, and bacterial quality of water where fish was caught (Bojanic*et al.*, 2009). And according to (Gram*et al.*, 1989), the count of Psychrotrophic bacteria increased after prolonged storage time of the fish on ice, so can uses as a good indicator for storage time.

Photo 1,2 shown that multiplex PCR was done to detect *Staphylococcus aureus* enterotoxins of isolated strains and there were nine strains have the ability to produce enterotoxins (39.1%) and found that the sea gene was amplified in 6 (26%) *S. aureus* strains giving product of (120 bp) and encoded enterotoxin A, seb gene was amplified in 2 (8.7%) S. aureus strains giving product of (478 bp) and encoded enterotoxin B, sec gene was amplified in 1 (4.3%) *S.aureus* strains giving product of (257 bp) and encoded enterotoxin C and sed gene was amplified in 1 (4.3%) *S.aureus* strains giving product of (317bp) and encoded enterotoxin D.

Our results were nearly similar to (Alibayov et al.,2014) who found 39.8% of strains demonstrated se genes , higher results were reported by (Aydin et al.,2011)who found that sea and seb genes were 53.9% and 53.9% respectively and (Jorgensen et al.,2005) who found that sec gene was 37.2%,

lower result was reported by (Zhang et al.,1998)who found that sed gene was 2.2%.

In table (3) the results showed that the *Staph.aureus* could be isolated from 23 (46%) from the examined samples. Our results are were lower than (Nancy et al.,2013) who mentioned that *Staph.aureus* was 90% in fish fillet samples and higher than (Mohamed et al.,2009) who reported that *Staph.aureus* was 3% in frozen fish fillet samples. Coagulase positive *Staphylococcus* is considered as an indicator of poor hygiene/handling procedures, *S. aureus* enterotoxins are considered one of the common causes of food poisoning worldwide, with outbreaks caused by mishandling of foods after heat treatment, Soriano et al. (2002) and Kadariya, (2014). Although, cooking destroys the bacteria, the toxin produced by *S. aureus* is heat stable and may not be destroyed even by heating, Ghosh, (2004). *Staphylococcus aureus* enterotoxins are the predominant cause of gastrointestinal symptoms observed during intoxications. *Staphylococcus aureus* is considered the third most important cause of disease in the world amongst the reported food-borne illnesses (Tamarapu*et al*., 2001).*S. aureus* entered into the foods during handling, processing or vending. It also due to the fact that it forms the normal microflora present on the skin and in the nose and throat of most healthy people. So contamination of foods with coagulase-positive *staphylococci* is largely as a result of human contact (Nester et. al., 2001).

In table (4) the results showed that the *E.coli* could be isolated from 5(10%) from the examined samples. our results agreed with (Gjerde,1976) found that incidence of *E.coli* 10% of the analyzed samples and lower than (Nancy et al.,2013) who mentioned that E.coli was 100% in fish fillet samples and higher than (Mohamed et al.,2009) who reported that E.coli was 5% in frozen fish fillet samples and E.coli would be easily atteunable by good manufacturing practices in case of fish fillet.

Table (5) show serotypes of *E. coli* out of the 5 strain isolated from frozen fish fillet and the serovars were :1(20%) isolated serovarO153 : H2 belonged to (EPEC); 2(40%) serovars O126 : H21 belonged to (ETEC) ; 1(20%) isolated serovarO55 : H7 and 1(20%) isolated serovarO121 : H7 belonged to (EHEC).

Such as EPEC, ETEC and EHEC is indicator of contamination and sewage pollution. Detection of ETEC in fish samples constitutes the main causes of food poisoning and hemorrhagic enteritis in man due to eating the improperly processed fish meals (Galal, 2013). EPEC induced watery diarrhea, vomiting and fever in infant and young children (Donenberg and Kaper, 1992). EHEC are very toxogenic that posses additional virulence factors, giving them ability to cause hemorrhagic colitis and hemolytic syndrome (Kobori et al.,2004).So, general sanitary measures were considered to be necessary as avoid contamination with exotic food born pathogens (only potable water should be used during the processing of fish fillet),ensuring the inactivation of pathogenic organisms by freezing process, at -18°C or lower and application of HACCP system in fish processing.

Humans are usually exposed to methyl mercury (Hg) through consumption of fish such as shark, swordfish and tuna. There is also bioaccumulation of Hg in fresh water fishes that live in contaminated lakes. Consumption of contaminated fish can pass the mercury to humans. Mercury at high doses can cause cerebral-palsy in children.

The mean value ± Stander error of mercury was 0.5992± 0.102 mg/kg as shown in table (6). These levels were nearly similar to the limit values for fish proposed by EOS (2005) was 0.50 mg/kg but lower than WHO (1990) indicated that Hg permissible limit is 2.0 ppm in sea foods.

Lead could contaminate water from industrial and agricultural discharges, high ways or motor traffic and from mine (Sorensen,1991). Lead residues could result in haematological, gastrointestinal and neurological dysfunction. Sever or prolonged exposure to pb may also cause chronic nephropathy, hypertension and reproductive impairment. pb inhibits enzymes, alter cellular calcium metabolism and slow nerve conduction (Friberg et al.,1985).

The mean value ± Stander error of lead was 0.1596± 0.037 mg/kg as shown in table (6).This result lower to those reported by Salah (2004) and Ahmed and Hussien (2004) who found mean level of pb 1.74 mg/kg in Tilapia nilotica. Our result nearly similar to labib et al.,(2008) who found that pb concentration ranged from 0.12 to 0.53 ppm in tilapia nilotica and Abou-Arab *et al.,* (1996) indicated that the FAO limit (1983) is 2.0 ppm and WHO (1990) indicated that pb permissible limit is 2.0 ppm in sea foods . Our result nearly similar to

the limit values for fish proposed by EOS (2005) was 0.10 mg/kg.

Cadmium can be found in all foodstuffs and particularly high amounts occur in organs of cattle, seafood and some mushroom species. Although the absorption of cadmium is low in GIT, it also has along biological half-life because it accumulates in the body. ATSDR, (1999) indicates that Cd may bioaccumulation in all levels of aquatic and terrestrial food chains. It accumulates largely in the liver and kidneys of vertebrates and not in muscle tissue (Sileo andBeyer 1985; Harrison Klaverkamp 1990 &Vos*et al*., 1990).

The mean value ± Stander error of cadmium was 0.059± 0.019 mg/kg as shown in table (6).Our result were lower than (El-Aziz and zaky,2010) who found level of cadmium in fish fillet 2.44±1.52 and agreed with Oehlenschlarger (1990) and Zauke*et al*., (1999) who found cadmium concentration below 0.1 mg/kg. Our result was lower than the limit values for fish proposed by EOS (2005) was 0.10 mg/kg.

The heavy metal concentrations in most fish fillet samples were well below the limits proposed for fish by various international standards and guidelines such as EU (2001), FAO/WHO (1989), MAFF (2000) and EOS (2005), and not dangerous to human health in that they are within the standards given by these regulations.

3. CONCLUSİON

Taking into consideration the obtained results it could be concluded that the total microbial load of frozen fish fillet was inacceptable and the high number of contaminated frozen fish fillet counts could pose a risk for human health after consumption of undercooked fish fillet. On the other hand The heavy metal concentrations in frozen fish fillet samples were well below the limits proposed for fish by various international standards and guidelines Regarding the daily intake and safety aspects, the examined fish fillet were safe for human consumption at least with regard to residual levels of lead (pb) cadmium (cd), and mercury (Hg) but a continuous monitoring of heavy metals in frozen fish fillet in markets is necessary to insure the prescribed worldwide limit.

4. REFERENCES

Abisoye, B. F., Ojo, S. K. S., Adeyemi, R. S., Olajuyigbe, O. O. 2011. Bacteriological assessment of some commonly sold fishes in Lagos metropolis market Nigeria. Prime J. Microbiol. Res. 1(2): 23-26.

Abou-Arab, A. A. K., Ayesh, A. M., Amra, H. A., Naguib, K. 1996.Characteristic levels of some pesticides and heavy metals in imported fish. Food Chem. 57(4): 487-492.

Ahmed, A. M., Hussein, M. M. 2004. Residual levels of some heavy metals in fish flesh and water from El-Manzala Lake, Egypt. J. King Saud Univ.Agricul. Sci.16(2): 187-196.

Alibayov, B., Zdeňková, K., Purkrtová, S., Demnerová, K., Karpíšková, R. 2014. Detection of some phenotypic and genotypic characteristics of Staphylococcus aureus isolated from food items in the Czech Republic. Annals Microbiol.64(4): 1587-1596.

Andreji, J., Stránai, I., Massányi, P., Valent, M. 2005. Concentration of selected metals in muscle of various fish species. J. Environmental Sci. Health, 40(4): 899-912.

APHA,1992. American Public Health Association,Compendium of methods for the microbiological examination of food. 3 31ed Ed., Washington , DC. New York.

Arannilewa, S. T., Salawu, S. O., Sorungbe, A. A., Ola-Salawu, B. B. 2005. Effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish (Sarotherodungaliaenus). African J. Biotechnol.4(8): 852-855.

Association of Official Analytical Chemists. 1980. Official methods of analysis (Vol. 534). W. Horwitz (Ed.). Arlington, VA, Washington DC: AOAC.

ATSDR, 1999. Agency for Toxic Substances and Disease Registry. ToxFAQs – Zinc. http://atsdr1.atsdr.cdc.gov:8080/tfacts60.

Aydin, A., Sudagidan, M., Muratoglu, K. 2011. Prevalence of staphylococcal enterotoxins, toxin genes and genetic-relatedness of food borne Staphylococcus aureus strains isolated in the Marmara Region of Turkey. Int.J. Food Microbiol.148(2): 99-106.

Bojanić, K., Kozačinski, L., Filipović, I., Fleck, Ž. C., Zdolec, N., Njari, B. 2009. Quality of sea bass meat during storage on ice. Meso, 11(1): 44-49.

Boyd, C. E. 1990. Water Quality in Ponds for Aquaculture, Alabama Agricultural Experiment Station, Auburn University, Auburn, Ala, USA.

Daviglus, M., Sheeshka, J., Murkin, E. (.2002. Health benefits from eating fish. Comments on Toxicol.8(4-6): 345-374.

Donenberg, M., Kaper, J.1992.EnteropathogenicE.coli. Infect. Immun. 60:3953.

Edwards, J. W., Edyvane, K. S., Boxall, V. A., Hamann, M., Soole, K. L. 2001. Metal levels in seston and marine fish flesh near industrial and metropolitan centres in South Australia. Marine Pollution Bulletin, 42(5):389-396.

Eklund, M. W., Peterson, M. E., Poysky, F. T., Paranjpye, R. N., Pelroy, G. A. 2004. Control of bacterial pathogens during processing of cold-smoked and dried salmon strips. Journal of Food Protection®, 67(2): 347-351.

El-Aziz, D. M. A., Zaky, Z. M. 2010. Assessment of heavy metal in local and imported filleted fish in Assiut City, Egypt. Assiut Veterinary Medical Journal, 56(126), 105-116.

Egyptian Organization for Standardization and Quality Control (2005) EOSQC.889-2/2005 for frozen fish, part 2 fish portions.

EU.2001. Commission Regulation as regards heavy metals, Directive, 2001/22/EC, No: 466.

FAO 1983.Compitation of legal limits for hazardous substances in fish and fishery products.FAO Fishery Circular 464: 5-100.

FAOn2010. The international fish trade and world fisheries.

FAO/WHO1989. Evaluation of certain food additives and the contaminants mercury, lead and cadmium, WHO Technical Report, Series No. 505.

FDA 2001.processing parameters needed to control pathogens in cold smoked fish. 979.

Friberg, L., Elinder, C. G., Kjellstrom, T., Nordberg, G. F. 1985. Cadmium and health: a toxicological and epidemiological appraisal. In Exposure, Dose and Metabolism (Vol. 1). CRC Press, Inc Boca Raton, FL.

Galal, H.M., Hakim, A.S., Sohad,M.Dorgham2013. Phenotypic And Virulance genes screening of Esherichia coli strains isolated from different sources in Delta Egypt. Life Sci. J.10 (2).

Gašpić, Z. K., Zvonarić, T., Vrgoč, N., Odžak, N., Barić, A. 2002. Cadmium and lead in selected tissues of two commercially important fish species from the Adriatic Sea. Water Res. 36(20): 5023-5028.

Ghosh, M., Mudgil, S.andGanguli, A. 2004.Microbiological quality of carrots used for preparation of fresh squeezed street vended carrot juices in India. J. Food Agric. Environ. 2: 143-145.

Gjerde, J. 1976. Bacteriological evaluation of frozen raw fish products. SvenskVetrinärtidning, 28(20): 911-917.

Gnanambal, M. E., Patterson, J. 2005. Biochemical and Microbiological Quality of Frozen Fishes Available in Tuticorin Super Markets. Fishery Technol. 42(1).

Gram, L., Oundo, J. O., and Bon, J. 1989. Storage life of Nile perch (Latesniloticus) in relation to temperature and initial bacterial load. Tropical Sci. 29 (4):221-236.

Harrison, S. E., Klaverkamp, J. F. 1990. Metal contamination in liver and muscle of northern pike (Esoxlucius) and white sucker (Catostomuscommersoni) and in sediments from lakes near the smelter at FlinFlon, Manitoba. Environmental Toxicol.Chem.9(7): 941-956.

Huss, H.H. 2003. Assessment and manegment of seafood safety and quality. Food Agriculture Organisation (FAO).Fisheries Techincal Paper444.Rome:FAO.

ISO/IEC 1999. International Standered Organization. Information technology encode of practice for information security management. Switzerland: International Organization for Standardization.

Järup, L. 2003. Hazards of heavy metal contamination. British medical bulletin, 68(1), 167-182.

Jay, J. M., Loessner, M. J., Golden, D. A. 2005. Modern food microbiology 7 th.

Jørgensen, H. J., Mørk, T., Caugant, D. A., Kearns, A., Rørvik, L. M. 2005. Genetic variation among *Staphylococcus aureus* strains from Norwegian bulk milk. Applied and environmental microbiology, 71(12), 8352-8361.

Jyh-Wei, S.,Yin-Hung, H. 2000.Investigation for Contamination of Parasite and Aerobic Bacteria in Frozen Tilapia Fillets in Taiwan. J. Food Drug Analysis. 8 (1). 51-56.

Kadariya, J., Smith, T. C., Thapaliya, D. 2014. Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. BioMedRes.Int. Article ID, 827965, 1-9.

Kobori, D., Rigobelo, E. C., Macedo, C., Marin, J. M., Avila, F. A. 2004. Virulence properties of Shiga toxin-producing Escherichia coli isolated from cases of bovine Mastitis in Brazil= Revue d'élevageet de médecinevétérinaire des pays tropicaux, 57(1-2).

Kok, T., Worswich, D., Gowans, E. 1996. Some serological techniques for microbial and viral infections. Mackie and McCartney Pract. Med. Microbiol.179-204.

Koutsoumanis, K., Nychas, G. J. E. 2000. Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. International J. Food Microbiol.60(2): 171-184.

Küçüksezgin, F., A. Kontas, O. Altay, E. Uluturhan, E. Darilmaz, E. 2006. Assessment of marine pollution in Izmir Bay: Nutrient, heavy metal and total hydrocarbon concentrations. Environ. Int. 32(1): 4151.

Labib, H. Y., Nassar, A., Ahmed, A., Shaker, A. 2008. Pollution of Nile fish by some heavy metals at Qena, Upper Egypt. In Proceedings of the Fourth International Conference for Development and Environment, Riyadh (pp. 18-20).

Mac Faddin, J. F. 2000. Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.

MAFF 2000. (Ministry of Agriculture, Fisheries and Food). Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1997. In Aquatic Environment Monitoring Report No. 52. Center for Environment, Fisheries and Aquaculture Science, Lowestoft, UK.

Mehrotra, M., Wang, G., Johnson, W. M. 2000. Multiplex PCR for Detection of Genes for*Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. J. Clinical Microbial.38 (3): 1032-1035.

Mhango, M., Mpuchane, S. F., Mpuchane, B. A. 2010.Incidence of indicator organisms, opportunistic and pathogenic bacteria in fish. African J. Food, Agriculture, Nutrit.Devel.10(10).

Mohamed W. S , EL-Mossalami E.I., Nosier Sh. M. 2009. Evaluation of Sanitary Status of Imported Frozen Fish Fillets and its Improvement by Gamma Radiation. J. Rad. Res. Appl. Sci. 2 (5):921-931.

NRDC2005.National resources defence council. Healthy milk, healthy baby: chemical pollution and mother's milk.

Nancy S. R., Ali M. A., Hosny A. A. 2013. Evaluation of Bacteriological Quality of some Imported Seafoods. SCVMJ, XVIII (2).

Nester, E.W., D.G. Anderson, C.E. Roberts, N.N. Pearsall, M.T. Nester 2001. Microbiology: A Human Perspective A human perspective. Microbiol 640-641.

Oehlenschlager, J. 2005. Identifying heavy metals in fish. In: Safety and Quality Issues in Fish Processing. Ed: Bremner, H.A. Woodhead Publishing Limited and CRC Press LLC. pp. 95-108.

Oelchlenschlarger, J. 1990. Phsphorous content of muscle of white fish types from the north Atlantic. Information fur dieFischwirstschaft, 37 (4): 149-158.

Olafsdottir, G., Lauzon, H. L., Jónsdóttir, R., Martinsdóttir, E. 2006. Multivariate quality prediction of cod (Gadusmorhua) and haddock fillets (Melanogrammusaeglefinus) stored under superchilling and temperature abusive conditions. In Proceeding of the 2nd Workshop on ‘‘Cold-Chain-Management’’. Icelandic Fisheries Laboratories, Skulagata (Vol. 4, p. 101).

Rall, V. L. M., Vieira, F. P., Rall, R., Vieitis, R. L., Fernandes, A., Candeias, J. M. G., Araújo, J. P. 2008. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. Vet. Microbiol.132 (3): 408-413.

Romeo, M., Siau, Y., Sidoumou, Z., Gnassia-Barelli, M. (1999): Heavy metal distribution in different fish species from the Mauritania coast. Sci. Total Environ. 232(3): 169-175.

Sanjee S. A.,Karim Md. E.2016. Microbiological Quality Assessment of Frozen Fish and Fish Processing Materials from Bangladesh.International journal of food science,

Saleh, E. A. 2004. Monitoring of some heavy metals residues in some fish. Alex. J. Vet. Sci. 21(1).

Satarug, S., Baker, J. R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P. E., Williams, D. J., Moore, M. R. 2003.A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol.letters 137 (1): 65-83.

Shah, D., Shringi, S., Besser, T., Call, D. 2009. Molecular detection of food borne pathogens, Boca Raton: CRC Press, In Liu, D. (Ed). Taylor and Francis group, Florida, USA, Pp. 369-389.

Sileo, L., and Beyer, W. N. 1985. Heavy metals in white-tailed deer living near a zinc smelter in Pennsylvania. J. Wildlife Dis. 21(3): 289-296.

Sorensen, E. M. 1991. Metal poisoning in fish. CRC press.

Soriano, J. M., Font, G., Moltó, J. C., Mañes, J. 2002. Enterotoxigenic staphylococci and their toxins in restaurant foods. Trends in Food Science and Technology, 13(2), 60-67.

Staniskiene, B., Matusevicius, P., Budreckiene, R., and Skibniewska, K. A. 2006. Distribution of heavy metals in tissues of freshwater fish in Lithuania. Polish J. Environ. Studies 15(4): 585.

Swanson, K. M. J., Busta, F. F., Peterson, E. H., and Johnson, M. G. 1992. Colony count methods. Compendium of methods for the microbiological examination of foods, 3: 75-95.

Tamarapu, S., McKillip, J. L., Drake, M. 2001.Development of a multiplex polymerase chain reaction assay for detection and differentiation of *Staphylococcus aureus* in dairy products. Journal of Food Protection®, 64(5), 664-668.

USDA/FSIS; United States Department of Agriculture Food Safety and Inspection Service (1998): Microbiology Laboratory Guidebook, 3 rd Ed., Washington, DC: USDA–FSIS.

USFDA (U.S. food and drug administration)-BAM (2001): Bacteriological Analytical Manual online, 8th edn., staphylococcus aureus.Chap.12.

Verbeke, W., Sioen, I., Brunsø, K., De Henauw, S., Van Camp, J. 2007. Consumer perception versus scientific evidence of farmed and wild fish: exploratory insights from Belgium. Aquaculture International, 15(2), 121-136.

Vos, G., Lammers, H., Kan, C. A. 1990. Cadmium and lead in muscle tissue and organs of broilers, turkeys and spent hens and in mechanically deboned poultry meat. Food Additives Contamin. 7(1): 83-91.

WHO 1990. Guidelines for sea food quality .2nd.ed.,V(1), Recommendation, WHO Geneva.

Yeasmin, T., Reza, M. S., Shikha, F. H., Khan, M. N. A., and Kamal, M. 2010. Quality changes in formalin treated rohu fish (Labeorohita, Hamilton) during ice storage condition. Asian J. Agricult. Sci. 2(4): 158-163.

Zauke, G. P., Savinov, V. M., Ritterhoff, J., and Savinova, T. 1999. Heavy metals in fish from the Barents Sea (summer 1994). Science of the Total Environ.227(2): 161-173.

Zhang, S., Iandolo, J. J., Stewart, G. C. 1998. The enterotoxin D plasmid of Staphylococcus aureus encodes a second enterotoxin determinant (sej). FEMS Microbiol. Letters 168 (2): 227-233.