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Microbiological Evaluation of Some Heat Treated Fish Products in Egyptian Markets

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Received: October 11, 2017; **Published:** December 13, 2017

Abstract

This study was conducted to confirm the bacterial conditions of fish products with E.O.S, and its hazards on public health. A total of 60 samples of fish products (smoked fish: herring and smoked salmon–semi cooked fish: fish finger and breaded shrimp) (15 of each) were collected from different retail markets for bacteriological examination. The average of APC, *Coliform*, *Escherichia coli*, Mould and yeast and *Staphylococcal aureus* counts (log_{10} cfu/g) were 4.17 ± 0.12, 2.92 ± 0.16, 2.19 ± 0.23, 3.96 ± 0.14 and 1.72 ± 0.21 for herring, respectively, 3.16 ± 0.19, 2.69 ± 0.13, 1.22 ± 0.16, 2.22 ± 0.18 and 1.06 ± 0.06 in smoked salmon, respectively, 2.78 ± 0.12, 2.02 ± 0.22, 1.59 ± 0.22, 2.14 ± 0.15 and 1.24 ± 0.24 in fish finger, respectively, and 2.60 ± 0.13, 2.33 ± 0.14, 1.46 ± 0.23, 1.96 ± 0.20, 0 ± 0 in breaded shrimp, respectively. The incidence of food poisoning organisms (*Salmonella* and *Listeria monocytogenes* and *Vibrio parahaemolyticus* also investigated and no one of them was isolated in the examined samples.

Keywords: *Salmon; Herring; Fish Finger; Breaded Shrimp*

Introduction

Fishes are known to be highly nutritious and excellent sources of animal protein, which is consumed by a larger percentage of the world's population because of its availability and palatability [1]. Research has shown that, fish smoking is the most widely practiced and recommended method of preservation where sophisticated equipment for more improved methods is lacking. Smoking of food is achieved by lowering of the water activity via application of gentle heat. The surface of food which will normally support most commensal organisms, is dried while the heat and chemicals inherent in the smoke deprives microbes of necessary growth factors [2]. Prior to smoking, various pre-treatments, such as salting and drying, and/or after treatments, e.g. cooking and marinating, are applied in the industry. However, smoking is not an absolute preserving method. For this reason, the quality of raw material, the concentration of salt, water activity of the fish, heat through the smoking process, the quantity of smoke, the way of packaging, hygienic circumstances and heat of storage have important effects in reducing the risk of deterioration [3,4]. Today smoking is no longer "necessary", but it remains popular for the flavor it gives to such fish as salmon, tuna, trout etc.

Also, The processes of battering and breading provide special functions in food products including improving the appearance of the products, increasing the texture, reducing the oil uptake during the frying process and increasing the shelf life of the coated products [5]. Battered and breaded fish products can undergo undesirable changes during frozen storage time due to microbial contamination from various sources and rapid spoilage as a result of protein denaturation [6] and lipid oxidation [7] leading to loss of quality.

Bacterial contamination in food often results in food spoilage as well as life-threatening health hazards like food poisoning [8].

Bacteriological examination is applied to evaluate the possible presence of microorganisms of public health significance and to give an impression about the hygienic quality of the fish. This includes temperature abuse and hygiene during handling and processing [9].

Estimation of APC is used as an index in standards, guidelines and specifications and considered more useful to estimate spoilage and the remaining shelf life of fish and fishery products [10].

In studies of seafood borne pathogens, four major pathogens have emerged as being of significant importance in terms of human health and disease. These include *Listeria monocytogenes, Vibrio parahaemolyticus*, *Staphylococcus aureus*, and *Salmonella* spp. [11]. *L. monocytogenes* has been isolated from fish and seafood products all over the world. *V. parahaemolyticus* is a human pathogen that occurs naturally in the marine environments and is frequently isolated from a variety of seafood including fish, shrimp, crab, lobster, scallop, and oyster [12]. This pathogen is a common cause of foodborne illnesses in many Asian countries, including Taiwan, China, and Japan, and is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States [13,14].

These facts greatly influenced the interest of this study which aimed at assessing the microbial load of retailed smoked fish (herring and salmon) and some battered and breaded fish products (fish fingers and breaded shrimp).

Materials and Methods

Collection of samples

A total of 60 random samples of fish products (smoked fish: herring and smoked salmon – semi cooked fish: fish finger and breaded shrimp) (15 of each) were collected from different supermarkets in Egypt. Each sample was kept in a separated sterile plastic bag and preserved in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay and examined as quickly as possible. The collected samples were subjected to the bacteriological examinations to evaluate their safety and fitness for human consumption.

Preparation of samples (APHA 2001)

Ten grams from each sample were weighted and stomached with 90 ml of 0.1% sterile buffered peptone water using stomacher (Seward stomacher 80 Biomasters, serial No 46464, England) to provide a dilution of 10-1. The homogenate was then allowed to stand for 15 minutes at room temperature. From the original suspension, one ml was transferred aseptically with sterile pipette into a test tube containing 9 ml of sterile buffered peptone water 0.1% and mixed well to produce a dilution of 10-2 from which further decimal serial dilutions were prepared.

Determination of Aerobic plate count [15].

Enumeration of *Coliform bacteria* and *Escherichia coli* [16].

Total Mould and Yeast Count [17].

Isolation and Enumeration of *Staphylococcus aureus* [18].

Detection and Enumeration of *Listeria monocytogenes* [19].

Isolation and identification of *V. parahaemolyticus* [20].

Results

It is evident from the result recorded in table 1 that APC in the examined samples varied from 3.54 to 4.97 with an average value of 4.17 ± 0.12 log cfu/g, 1.00 to 3.92 with an average value of 3.16 ± 0.19 log cfu/g, 2.04 to 3.72 with an average value of 2.78 ± 0.12 log cfu/g and 2.00 to 3.36 with an average 2.60 ± 0.13 log cfu/g for the examined samples of herring, salmon, fish finger and breaded shrimp, respectively. Table 2 showed that the mean ± SE of *coliform* and *E. coli* count of examined samples of herring, salmon, fish finger and breaded shrimp were 2.92 ± 0.15 and 2.19 ± 0.23, 2.69 ± 0.13 and 1.22 ± 0.16, 2.02 ± 0.22 and 1.59 ± 0.22 and 2.33 ± 0.14 and 1.46 ± 0.23, respectively. Results achieved in table 3 indicated that the mean ± SE of moulds and yeast count of examined samples of herring,

salmon, fish finger and breaded shrimp were 3.96 ± 0.14, 2.22 ± 0.18, 2.14 ± 0.15 and 1.96 ± 0.20, respectively. It is evident from the results recorded in table 4 that the mean ± SE of *Staphylococcal aureus* count of examined samples of herring, salmon, fish finger and breaded shrimp were 1.72 ± 0.21, 1.06 ± 0.06, 1.24 ± 0.24 and 0, respectively. Table 5 showed that the percentage and occurrence of *Salmonella, Listeria monocytogenes* and *Vibrio parahaemolyticus* in examined samples of herring, salmon, fish finger and breaded shrimp based on their contamination were 0%, 0%, 0% and 0% of all investigated microorganisms respectively. Moreover, the results in table 6 showed that 100% and 100% of herring, salmon respectively, were unaccepted according to E.O.S (2005, 288) [21]. The results achieved in table 7 showed that 40%, 33%, 3.33% of fish finger and breaded shrimp, respectively were unaccepted according to E.O.S (2005, 3495) [21].

No. of positive samples %	**Smoked fish**		**Semi-cooked**	
	Herring 15 100	**Salmon 15 100**	**Fish finger 15 100**	**Breaded shrimp 15 100**
Mini.	3.54	1.00	2.04	2.00
Maxi.	4.97	3.92	3.72	3.36
Mean	4.17	3.16	2.78	2.60
SE	0.12	0.19	0.12	0.13

Table 1: *Statistical analytical results of Total aerobic plate count contamination in fish products samples (log cfu/g) mean ± SE*.*

No. of positive samples %			**Smoked fish**				**Semi cooked**		
	Herring		**Salmon**		**Fish finger**		**Breaded shrimp**		
	Coliforms 14 93	***E. coli* 4 27**	**Coliforms 6 40**	***E. coli* 6 40**	**Coliforms 11 73**	***E. coli* 5 33**	**Coliforms 5 33**	***E. coli* 5 33**	
Min.	2.04		1.70	2.18	1.00	0.04	1.00	2.04	1.00
Max.	3.80		2.60	2.97	2.00	2.79	2.18	2.79	2.00
Mean	2.92		2.19	2.69	1.22	2.02	1.59	2.33	1.46
SE	0.16		0.23	0.13	0.16	0.22	0.22	0.14	0.23

Table 2: *Statistical analytical results of Coliforms and E. coli counts contamination in fish products samples (No = 15 each) log cfu/g; mean ± SE.*

No. of positive samples %	**Smoked fish**		**Semi- cooked**	
	Herring 15 100	**Salmon 10 67**	**Fish finger 11 73**	**Breaded shrimp 10 67**
Mini.	3.11	1.40	0.79	1.15
Maxi.	4.72	2.97	2.63	3.11
Mean	3.96	2.22	2.14	1.96
SE	0.14	0.18	0.15	0.20

Table 3: *Statistical analytical results of Mould and yeast count contamination in fish products samples (No = 15 each) log cfu/g; mean± SE.*

No. of positive samples %	Smoked fish		Semi- cooked	
	Herring 12 80	Salmon 5 33	Fish finger 2 13	Breaded shrimp 0 0
Mini.	0.70	1.00	1.00	-
Maxi.	2.78	1.30	1.48	-
Mean	1.72	1.06	1.24	-
SE	0.21	0.06	0.24	-

Table 4: *Statistical analytical results of Staphylococcus aureus count contamination in fish products samples (No = 15 each) log cfu/g; mean± SE.*

Isolates	Smoked fish				Semi cooked			
	Herring		Salmon		Fish finger		Breaded shrimp	
	No	%	No	%	No	%	No	%
Salmonella spp.	-	-	-	-	-	-	-	-
Listeria monocytogenes	-	-	-	-	-	-	-	-
Vibrio parahaemolyticus	-	-	-	-	-	-	-	-

Table 5*: Frequency and percentage occurrence of bacterial isolates of fish products samples.*

	Standards	Herring				Salmon			
		Acceptable		Non Accept		Acceptable		Non Accept	
		No/15	%	No/15	%	No/15	%	No/15	%
APC	≤ 100000	15	100	0	0	15	100	0	0
Coliforms	≤ 10	1	7	14	93	9	60	6	40
Moulds	Free	0	0	15	100	0	0	15	100
E. coli	Free	11	73	4	27	9	60	6	40
Listeria, mono	Free	15	100	0	0	15	100	0	0
Salmonella	Free	15	100	0	0	15	100	0	0
Staph aureus	Free	4	27	11	73	10	67	5	33
Vibrio para.	Free	15	100	0	0	15	100	0	0

Table 6*: Acceptability of the examined samples of smoked fish samples according to EOSQC (2005/288).*

	Standards	Herring				Salmon			
		Acceptable		Non Accept		Acceptable		Non Accept	
		No/15	%	No/15	%	No/15	%	No/15	%
APC	≤ 100000	15	100	0	0	15	100	0	0
Coliforms	≤ 100	6	40	9	60	10	67	5	33
Moulds	Free	4	27	11	73	5	33	10	67
E. coli	Free	10	67	5	33	10	67	5	33
Listeria, mono	Free	15	100	0	0	15	100	0	0
Salmonella	Free	15	100	0	0	15	100	0	0
Staph aureus	Free	13	87	2	13	15	100	0	0
Vibrio para.	Free	15	100	0	0	15	100	0	0

Table 7: *Acceptability of the examined samples of smoked fish samples according to EOSQC (2005/3495).*

Discussion

Fish is a reservoir of large number of microorganisms; one of the major factors contributing to poor quality of the fish in retail trade is unhygienic handling and storage leading to off smell, physical damage and contamination with dirt and objectionable microorganisms (Sugumar., *et al.* 2004). Eyo [22], stated that microbial action has been known to play a large part in the spoilage of fish. Bacterial spoilage is characterized by softening of the muscle tissue and the production of slime and offensive odours.

The aerobic plate count

The aerobic plate count (APC) is an important factor for evaluation of microbial quality assessment in food products and is an indicator of the overall degree of microbial contamination of foods [20]. APC does not measure the entire bacterial population but rather the number bacteria growth in the presence of oxygen (aerobically) and at medium range (mesophilic) temperatures.

Table 1 revealed that, the mean value of aerobic plate counts APC (log^{10} cfu/g) mean ± SE in the examined smoked fish (smoked fish (Herring – Salmon) and semi-cooked fish products (Fish finger -- Breaded shrimp) were (4.17 ± 0.12--3.16 ± 0.19) and (2.78 ± 0.12--2.60 ± 0.13) respectively. Higher findings observed in Khater and Farag [23] who found the APC in herring and salmon paste samples were 5.35 ± 0.23 and 5.34 ± 0.68 respectively, also Ibrahim., *et al*. 2014 found the APC in smoked fish was 2.06×10^6 cfu/g. The bacterial load were found to be higher in the smoked fish samples which might be due to secondary contamination during the time of handling as well as storage of fishes in ice made from contaminated water, poor hygiene and sanitation condition of processing [24]. Smoking helps in inhibiting the activities of microorganisms, however, when smoking process not properly carried out, microbial growth and activities still continue, leading to the deterioration of the fish Thus, TAC is considered a quality indicator for food. Although there is not direct correlation between this and the presence of pathogenic microorganisms, TAC is an indicator of the shelf-life of products, and also the potential for growth of the microorganism that is present [25]. Our lower findings observed in fish finger and breaded shrimp may be due to adding garlic and pepper powder and other spices caused to reduce the bacterial count in fish fingers due to their antibacterial role. Higher results were seen in Ibrahim-Hemmat., *et al*. [26] who found the APC of fish finger was $8.33 \times 10^4 \pm 1.04 \times 10^4$ cfu/g.

Coliform and *E. coli* count

The results recorded in table 2 revealed that, the mean value of *coliforms* and *Escherichia coli* count in (smoked fish (Herring -- Salmon) and semi cooked fish products (Fish finger -- Breaded shrimp) were 2.92 ± 0.16, 2.19 ± 0.23 for herring, 2.69 ± 0.13, 1.22 ± 0.16 for salmon and 2.02 ± 0.22, 1.59 ± 0.22 for fish finger and 2.33 ± 0.14, 1.46 ± 0.23 for breaded shrimp. The *Coliform* counts were low in semi-cooked fish products; this may be due to the attained temperature for frying was sufficient to kill vegetative bacteria.

Data presented in table 2 showed that, the mean value of *coliform* count came in parallel with those of [27-29]. Munce [30] stated that presence of coliform in food has been linked with the practice of inadequate hygienic measure, mishandling, improper storage and use of dirty water during marketing and all unhygienic condition of the shops.

Mould and yeast count

The incidence of mould in fish could be attributed to improper sanitation during catching, handling, processing, salting, storage, transportation, distribution and marketing of fish. Contamination with a variety of mould species resulted in undesirable changes of fish and rendering it unfit for marketing and increase the risk of infection with respective disease to consumers as a probable result of aflatoxins production by some fungal strains. The results recorded in table 3 revealed that, the mean value of mould and yeast counts (log_{10} cfu/g) mean ± SE in the examined smoked fish (Herring -- Salmon) and semi-cooked fish products (Fish finger -- Breaded shrimp) were 3.96 ± 0.14, 2.22 ± 0.18, 2.14 ± 0.21 and 1.96 ± 0.20, respectively. Cold-smoked fish are not cooked, because the temperature generally does not exceed 43°C. Therefore, the most common causes of spoilage in smoked fish are mold growth. The count of molds and yeast in Herring and Salmon respectively. Similar results have been reported by Tadros-Safaa [31] who found that the mean value of total mould count

was 7.5 X 10^2 ± 2.4 X 10^2/g of smoked fish. Nearly similar findings obtained by Ibrahim-Hala [32] who reported that the mean value of the total mould count/g of smoked fish was 3.5 X 10^3 ± 1.3 X 10^3. Also El-Sayed [33] reported that the mean value of total mould count/g of smoked fish was 15.3 X 10^3.

***Staphylococcus aureus* count**

Staphylococcus aureus is a major cause of food poisoning due to ingestion of enterotoxins [34]; the ability to produce such enterotoxins in food is more likely when competing microorganisms were absent [35]. It is evident from the results recorded in table 4 revealed that the incidence of *Staphylococcus aureus* were 80, 33, 13 and 0% in the examined smoked fish (Herring -- Salmon) semi-cooked (fish finger and breaded shrimp) with an average 1.72 ± 0.21 and 1.06 ± 0.06 in herring and smoked salmon, respectively. While in examined semi-cooked fish products fish finger was 1.24 ± 0.24 and breaded shrimp samples were free from coagulase positive *Staph. aureus*. Also our results were in agreement with Adeyeye., *et al.* [36] who found the mean *S. aureus* count of smoked fish 1.1 ± 102 to 3.8 ± 10^2 cfu/g whereas Zaki [37] recorded 3 log cfu/g *Staphylococcus aureus* count in smoked fish which was higher than our results. The presence of *Staphylococcus aureus* in smoked samples can be attributed to post-processing Contamination. Our results were agreed with those of [38,39] who found that all examined shrimp samples were free from coagulase positive *S. aureus*.

Prevalence of food poisoning organisms (*Salmonella* and *Listeria monocytogenes* and *Vibrio parahaemolyticus*

The results recorded in table 6 revealed that non of the three food poisoning organisms were detected in the examined fish products samples.

Salmonella

Salmonella was not detected in the samples analysed in this study, which was in agreement with previous studies [40] in seafood products. Meanwhile, disagreed with those of Soliman., *et al*. [27], Younis [41], who isolated *Salmonella* from fried fish, shrimp.

Vibrio-parahaemolyticus

V. parahaemolyticus is an indigenous bacterium in the marine environment and can also grow in 1 - 8% salt [42,43]. *Salmonella* spp. and *V. parahaemolyticus* in aquaculture products mainly originates of hygiene and sanitation. But sometimes, incidence of these bacteria in fish may occur due to external contamination. Fortunately no presence of pathogenic *Vibrio-parahaemolyticus* were found in all inspected fish products samples.

Listeria monocytogenes

In the present study, *L. monocytogenes* not detected in all examined samples. Similar results observed in Jalali and Abedi [44] they don't found *L. monocytogenes* in 85 samples of fresh and frozen fish and shrimp analyzed. *L. monocytogenes* contamination of seafood varies with product category. Jorgensen and Huss [45] demonstrated that the highest prevalence of *L. monocytogenes* is in cold-smoked fish (34% - 60%), whereas the lowest is in heat-treated and cured seafood (4% - 12%). In general, *L. monocytogenes* is not usually found on fish captured from open waters. However, contamination may take place long before the fish raw material reaches retail trade or processing factories. Potential sources of *L. monocytogenes* on fishing vessels include contamination from water and ice, soiled surfaces, and boxes as well as from human and avian sources. As *L. monocytogenes* is commonly found in coastal waters and in surface waters of lakes, fish captured or cultivated in these waters may possibly carry this microorganism [46].

Table 6 showed that 100%, 100% were unaccepted based on their moulds and yeasts count/g according to E.O.S (2005) [21] of examined samples of` herring and smoked salmon respectively. Results achieved in table 7 indicated that 73% and 67% of the examined fish finger and breaded shrimp samples were unaccepted based on their moulds and yeasts count/g according to E.O.S. (2005) [21,47].

Conclusion

Finally, the study concluded that smoked fish, which are ready for immediate human consumption, have unacceptable microbial quality. However, they may consider of high-risk due to fungal toxins hazards.

So, special attention should be taken from competent authorities and food business operators. Moreover, consumers are increasingly aware of the danger of pathogens in RTE fish. Also, the present study proved that semi-cocked are considered of public health hazard due to the presence of considerable percentages of *coliforms*.

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Volume 12 Issue 3 December 2017