COMPARATIVE STUDY BETWEEN RAW AND COOKED FISH SOLD IN ASSIUT CITY ON THE INCIDENCE OF SOME FOODBORNE PATHOGENS

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ABSTRACT

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The present investigation was carried on 90 random samples (30 of each) or raw and 60 cooked (grilled and fried,each 30) fish which obtained from different fish market and restaurants in Assiut city. These samples were examined bacteriologically by standard procedures for determination of Aerobic plate count, and counts of coliform, faecal coliform, *E.coli and Staph aureus* where the mean values of these microorganisms in raw fish were: 44.6 x 10^5 ± 22.7x10^5, 56.6x10 ± 7.6 x 10, 0.8 x 10±0.1x10, 0.7 x 10 ±0.1 x 10 and 32.8 x 10 ± 14.9 x 10 /g respectively. Wherease the corresponding mean values of grilled fish were: 22.3 x 10^5 ± 5.6 x 10^5, 48 x 10 ± 7.6 x 10, 0.6 x 10±0.08 x 10,0.7 x 10±0.2 x 10 and 21.5 x 10±12.9 x 10/g respectively. While the mean values in fried fish were: 2.1 x 10^5±0.7 x 10^5,19.7 x 10 ± 6.7x 10, 0.4 x 10 ± 0.05 x 10, 0.5 x 10 ± 0.2 x 10 and 5.5 x 10 ± 3.5 x 10 /g respectively. Some foodborne pathogens as *E.coli, Staph aureus C.perfrengens, Listeria monocytogenes and Aeromonas* spp, could be isolated from raw fish in incidence of 13.3, 30, 46.7, 10 and 73.3% respectively while that in grilled fish was 16.7, 20, 20, 6.7 and 40% respectively. As for fried fish the incidence was 6.7, 13.3, 10, 10 and 30% respectively. *Samonella* failed to be recovered from all examined samples. The public health importance of the recovered microorganisms as well as some recommended measures for improving the quality of such products were discussed.

Key words: *Raw fish, cooked fish, fish market, food borne pathogen.*

دراسة مقارنة بين السمك الطازج والمطهى المباع في مدينة أسيوط على تواجد بعض الميكروبات الممرضة المنقولة بالغذاء

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أجريت هذه الدراسة على ٩٠ عينة من السمك الطازج والمطهى (المقلي والمشوى) بواقع ٣٠ عينة من كل نوع من محلات بيع الأسماك المختلفة وعدد من المطاعم الموجودة في مدينة أسيوط حيث تم الفحص البكتريولوجي بهدف تعيين العدد الكلي للميكروبيات الهوائية، ميكروبات القولون، ميكروبات القولون البرازي، ميكروبات الأشريكية القولونية وميكروبات المكور العنقود الذهبي حيث كانت متوسطات هذه الميكروبات في السمك الطازج كالآتي: ٤٤.٦ × ١٠^{٥} ± ٢٢.٧ × ١٠^{٥} ، ٥٦.٦ × ١٠ ± ٧.٦ × ١٠ ، ٠.٨ × ١٠ ± ٠.١ × ١٠ ، ٠.٧ × ١٠^{٥} ± ٠.١ × ١٠^{٥} و ٣٢.٨ × ١٠ ± ١٤.٩ × ١٠ /جرام بالترتيب. أما في السمك المشوي فكانت كالآتي: ٢٢.٣ × ١٠^{٥} ± ٥.٦ × ١٠^{٥} ، ٤٨ × ١٠ ± ٧.٦ × ١٠ ، ٠.٦ × ١٠ ± ٠.٠٨ × ١٠، ٠.٧ × ١٠ ± ٠.٢ × ١٠ و ٢١.٥ × ١٠ ± ١٢.٩ × ١٠ /جـــرام

بالترتيب. بينما كانت المتوسطات في السمك المقلي كالاتي: ٢.١ × ١٠^٥ ± ٠.٧ × ١٠^٥ ، ١٩.٧ × ١٠ ± ٦.٧ × ١٠ ، ٠.٤ × ١٠ ± ٠.٠٥ × ١٠ ، ٥.٠× ١٠ ± ٠.٢ × ١٠ و ٥. ٥ × ١٠ ± ٣.٥ × ١٠ /جرام بالترتيب. كما تم عزل بعض الميكروبات الممرضة: ميكروبات الأيشاريشياكولاي، العنقود الذهبي، الكلوستريديم بيرفيرنجيز، الليستريا مونوسيتوجين وميكروبات الأيرومونس بنسب مختلفة كالآتي: ١٣.٣ ، ٣٠ ، ٤٦.٧ ، ١٠ و ٧٣.٣ % بالترتيب من عينات السمك الطازج التي تم فحصها أما في السمك المشوي فقد كانت النسب كالآتي: ١٦.٧ ، ٢٠ ، ٢٠، ٦.٧ و ٤٠% على التوالي.بينما كانت النسب في السمك المقلي كالآتي: ٦.٧ ، ١٣.٣ ، ١٠ ، ٣.٣ و ٣٠% على التوالي ولقدا دلت النتائج على عدم وجود ميكروب السالمونيلا في جميع العينات التي تم فحصها. هذا وقد تمت مناقشة الأهمية الصحية لهذه الميكروبات ومدى خطورتها على الصحة العامة كذلك الطرق المقترحة للحد من هذه الخطورة.

INTRODUCTION

Fish are very important source of protein specially in Egypt where animal protein is insufficient to meat the requirements of the population. They have long been regarded as nutritive and highly desirable food due to its contribution of high quality animal protein, its exceptional riches in calcium and phosphorus and its generous supply of B-complex (Mutkoski and Schurer, 1981).

Quality of fish is often more difficult to control due to variations in species, sex, age, habitats and action of autolytic enzymes as well hydrolytic enzymes of microorganisms on the fish muscle (Venugopol, 2002). Safety of ready-to-eat fish meals with reference to bacterial contamination is usually concerned with possibility of the food infection and intoxication.

In general, when a healthy fish is caught, the fish is sterile as its immune system prevents bacteria to proliferate easily whereas after death the fish's immune system collapses allowing easly penetration of microorganisms into the flesh (Huss, 1995). This peneteration increase in case of fish caught from polluted area where there are high densities of bacteria (Howgate, 1998). So, that many investigators convinced that fish from polluted environment may be passive carriers of bacteria pathogenic to man (Varnam and Evans, 1991).

Furthermore, pathogenic bacteria are naturally present in aquatic environment (*Clostridium, Aeromonas)* and the general environment (*L.monocytogenes*). Other microorganisms are of animal / human reservoir *(Salmonella, E. coli*), thus there is always a possibility that these microorganisms may be passed on to the raw material during production and processing (Huss *et al.,* 2000).

Contamination is a very important aspect as this is the mode that most unwanted microorganisms may be transmitted onto seafood and other products. These unwanted microorganisms may access food processing environments through raw materials, personel or mobile equipment or through pests and some pathogens may even become established in the processing plant and from niches where they can survive for long periods of time (Reij *et al.,* 2003). Transfer of microorganisms by personnel particulary from hands, is of vital importance (Chen *et al.,* 2001, Montville *et al.,* 2001; Bloom field, 2003). During handling and preparation, bacteria are transferred from contaminated hands of food workers to food and subsequently to other surfaces (Montville *et al.,* 2002). Water is also a vehicle for transmission of may agents of diseases (Kirby *et al.,* 2003).

The degree of cooking employed further effects on the number and the types of microorganisms. Moreover, organisms normally associated with raw fish are not heat resistant and are destroyed during heat process. Heat resistant types of organisms may be introduced with spices or other ingredients (Nickelson and Finne, 1984). Therefore morphological quality as well as sources of contamination of such meals have been studied by many researchers.

The purpose of this investigation was to determine the bacteriological status (aerobic count, coliforms, faecal coliforms, *E.coli, Staph aureus, Aeromonas* spp. *Salmonella* spp.*, Listeria monocytogenes and Clostridium perfringens)* of raw and cooked (fried and

grilled) fish and monitoing the public health importance of the isolated organisms as well as some of the recommended measures for improving the quality of such products.

MATERIALS and METHODS

Collection of samples:

A total of 90 random samples of fish represented by 30 raw freshwater and 60 ready-to-eat (grilled and fried, each 30) fish were collected from fish markets and restaurants with different sanitation levels in Assiut city. All the collected samples were then transferred to the laboratory under complete aseptic conditions without undue delay where they were prepared and examined.

Sampling (Scott *et al.,* 1992):

Flesh samples were taken from the left hand side of each fish in the anterior dorsal region. For raw fish, the skin was rinsed with 95% ethanol and flamed. For all collected samples, the skin was removed and the underlying flesh was aseptically transferred into a clean separate sterile mortar. Each sample was mixed well then prepared for bacteriological examination:

Preparation of samples:

To 25gm of each samples, 225 ml of sterile0.1%peptone water were added and thoroughly mixed using sterile blender for approximately 2 min.to obtain a dilution of 1/10, then decimal dilutions were prepared as recommended by APHA (1992). The prepared dilutions and samples were subjected to the following examinations**:**

A-Enumeration procedures:

1- Aerobic plate count (APC): The technique recommended by APHA (1992) using surface plating method was used.

2- Colifrom, faecal coliform and ***E.coli*** count (MPN/g): According to the technique out lined by AOAC (1990).

3- ***Staph aureus*** count: The surface plating technique of Baird –Parker ager plates as described by APHA (1992) was followed.

B- Isolation procedures:

1-Detection of *Salmonella* spp:According to the method recorded byAPHA (1992).

2-Isolation of *Listeria* spp.:The technique recommended by Grey and killinger 1966.

3-Isolation of *Clostridium perfringens*:This was done according to the technique adopted by Angeloti *et al.* (1967).

4- Isolation of *Aeromonas* spp.:The technique was done as described by Okrend *et al.* (1987), and Ahmed *et al.* (1991).

5- Isolation of *Staph.aureas*:Was carried out using Mannitol Salt agar as recommended by Feingold and Martin (1982).

RESULTS

Table 1: Statistical values of aerobic plate count /gm of the examined raw, grilled and fried fish samples (n = 30 of each).

Types of samples	Positive samples		Minimum	Maximum	Mean	SE	P. value
	No	%					
Raw fish	30	100	$88x10^3$	$52x10^6$	$44.6x10^5$	$22.7x10^5$	< 0.001 ***
Grilled fish	30	100	$25x10^3$	$93x10^5$	$22.3x10^5$	$5.6x10^5$	< 0.001 ***
Fried fish	30	100	$22x10^3$	$7x10^5$	$2.1x10^5$	$0.7x10^5$	N.S

Table 2: Statistical values of coliform count (MPN/gm) of the examined raw, grilled and fried fish samples (n = 30 of each).

Types of samples	Positive samples		Minimum	Maximum	Mean	SE	P. value
	No	%					
Raw fish	30	100	$2.1x10^2$	$1.1x10^3$	56.6x10	7.6x10	< 0.001 ***
Grilled fish	30	100	$1.5x10^2$	$1.1x10^3$	48x10	7.6x10	< 0.001 ***
Fried fish	30	100	9.1	$1.1x10^3$	19.7x10	6.7x10	N.S

Table 3: Statistical values of coliform count (MPN/gm) of the examined raw, grilled and fried fish samples (n = 30 of each).

Types of samples	Positive sample		Minimum	Maximum	Mean	SE	P. value
	No	%					
Raw fish	28	93.3	3	1.5x10	0.8x10	0.1x10	< 0.001 ***
Grilled fish	26	86.7	3	1.5x10	0.6x10	0.08x10	< 0.05*
Fried fish	21	70	3	7.3	0.4x10	0.05x10	N.S

Table 4: Statistical values of *E.coli* count (MPN/gm) of the examined raw, grilled and fried fish samples (n = 30 of each).

Types of samples	Positive samples		Minimum	Maximum	Mean	SE	P. value
	No	%					
Raw fish	4	13.3	7.3	20	0.7x10	0.1x10	N.S
Grilled fish	5	16.7	3.6	14	0.7x10	0.2x10	N.S
Fried fish	2	6.7	3.6	7.3	0.5x10	0.2x10	N.S

Table 5: Statistical values of *Staph aureus* count / gm of the examined raw, grilled and fried

fish samples (n = 30 of each).

Types of samples	Positive samples		Minimum	Maximum	Mean	SE	P. value
	No	%					
Raw fish	6	20	8 x 10	1 x 10^3	32.8x10	14.9x10	N.S
Grilled fish	4	13.3	5 x 10	6 x 10^2	21.5x10	12.9x10	N.S
Fried fish	2	6.7	2 x 10	9 x 10	5.5x10	3.5x10	N.S

N.S.: Non significant ***:** significant ****:**moderatly significant *****:**highly significant

Table 6: Incidence of the isolated microorganisms from the examined raw, grilled and fried fish samples.

Organisms	Type of Samples						Total	
	Raw fish		Grilled fish		Fried fish			
	N/30	%	N/30	%	N/30	%	N/30	%
E.coli	4	13.3	5	16.7	2	6.7	11	12.2
Staph aureus	9	30	6	20	4	13.3	19	21.1
C.perfringens	14	46.7	6	20	3	10	23	25.6
Listeria spp	8	26.7	5	16.7	3	10	16	17.8
L.monocytogenes	3	10	2	6.7	1	3.3	6	6.7
Aeromonas spp	22	73.3	12	40	9	30	43	47.8
A.hydrophila	12	40	6	20	3	10	21	23.3
A.caviae	7	23.3	4	13.3	5	16.7	16	17.8
A.sorbia	3	10	2	6.7	1	3.3	6	6.7
Salmonella spp	0	0	0	0	0	0	0	0

DISCUSSION

From the summarized results given in Table 1 it is evident that all the examined raw, grilled and fried fish samples (100%) contained viable bacteria. In raw fish, the aerobic plate count (APC) varied from 88 x 10^3 to 52 x 10^6 with a mean value of 44.6 x 10^5 ± 22.7 x 10^5 cfu /gm while that of grilled fish ranged from 25x10^3 to 93 x 10^5 with a mean value of 22.3 x 10^5 ± 5.6 x 10^5 cfu/gm/. As for fried fish, their mean APC was 2.1x10^5 ± 0.7 x 10^5 cfu/gm with a minimum of 22x10^2 and a maximum of 7x10^5.

Correlation between the aerobic plate count and types of fish samples examined recorded in Table 1 revealed that there was a high significant difference in the mean aerobic plate count between each of raw, grilled and fried fish samples.

Lower counts of aerobic bacteria were enumerated in raw fish by Surkewicz *et al.* (1968), Thabet (1972), Farouk (1989) and Mahmoud (1999) who recorded an average values of 2.5 x 10^4, 15 x 10^2, 10^4 and 3x10^3 cfu/gm, respectively. On the other hand Yousef *et al.* (1985) and Morshidy (1992 a) reported higher counts represented by a mean values of 78.7 x 10^5 and 10 x 10^6 cful/gm raw fish. As for ready – to- eat fish, Hefnawy (1990) cited a mean APC of 22.2 x 10^2 / gm of fried fish which seem to be lower than the obtained results whereas Eldaly and Ibrahim (1987) reported higher mean APC which

were 2 x 10^6 and 9 x 10^6 cfu/gm of the examined grilled and fried fish respectively.

However, fish and shellfish of good quality will have counts less than 1 x 10^5 / gm of tissue at 20ºC. High counts should be considered an evidence of a potentially hazardous situation (FAO, 1992).

Coliforms as recorded in Table 2 were existed in all the examined (100%) raw fish samples in number varied from 2.1 x 10^2 to 1.1 x 10^3 with a mean MPN value of 56.6 x 10 ± 7.6 x 10 /gm. In this respect Surkewicz *et al.* (1968) reported that the MPN of coliforms was less than 10/gm raw fish, in addition lower coliform counts were recorded by Farouk (1989) and El-Sayed (1991) who reported an average MPN value of 30 and 2.5 x 10/gm, respectively. On the other hand, higher findings (4.47 x 10^4, 14 x 10^3, 5.8x10^2, 4.47 x 10^4 and 6.6 x 10^3/ gm) were reported by Morshidy and Hafez (1986), Abdel –Galil *et al.* (1988), Naser (1991), Morshidy (1992b) and Mahmoud (1999), respectively.

Regarding ready-to-eat fish, all the examined samples (100%) had coliforms where the level of contamination in grilled fish ranged from 1.5x10^2 to 1.1 x 10^3 with a mean MPN value of 48 x 10 ± 7.6 x 10/gm whereas fried fish contained coliforms at a level varied from 9.1 to 1.1x10^3 with a mean MPN value of 19.7 x 10 ± 6.7 x 10/gm. Eldaly and Ibrahim (1987) recorded a mean coliform count of 2x10^4 and 6 x 10^2 / gm of grilled and fried fish samples examined, respectively, However, a high significant difference in the mean MPN of coliforms could be detected between raw and fried fish as well as between grilled fish and fried ones (Table 2).

Furthermore, in table 3 faecal coliforms were detected in the examined raw and ready-to-eat fish samples. Majority (93.3%) of the positive raw fish samples were contaminated with these organisms in counts ranged from 3 to 1.5 x 10 with a mean MPN value of 0.8 x 10 ± 0.1 x 10 /gm. Moreover, 86.7 and 70% of the examined grilled and fried fish samples had a mean MPM values of 0.6 x 10 ± 0.08 x 10 and 0.4 x 10 ± 0.05 x 10/gm with minimum of 3 and 3 and a maximum of 1.4 x 10 and 7.3 x 10/gm, respectively. A highly significant difference in the mean MPN of faecal coliforms was noticed between raw and fired fish and this variation was significant between grilled and fried fish.

The presence of coliforms in food indicates a potable faecal source of contamination. Their significance in food deponds upon the circumstances to with the food has been exposed and their presence in great number may raise the public health hazard (National Academy of Sciences 1995).

As for *E.coli*, Table 4 verify that 13.3, 16.7 and 6.7% of the examined raw, grilled and fried fish samples contained variable numberS where their MPN values were 0.7 x 10 ± 0.1 x 10, 0.7 x 10 ± 0.2 x 10 and 0.5 x 10 ± 0.2 x 10 /gm, respectively. Most of the examined fish samples had MPN was < 3/gm. No significant difference in the mean MPN of *E.coli* was noticed between the three examined fish samples. Eldaly and Ibrahim (1987) recorded a mean MPN values of 2x10^2 and 48/gm of the examined grilled and fired fish which seem to be higher than that obtained in the present study.

The findings outlined in Table 5 declared that *Staph aureus* was existed in variable numbers in 20, 13.3 and 6.7% of the examined raw, grilled and fired fish samples respectively whereas the remainder of the samples contained non detectable levels. The mean *Staph aureus* count values were 32.8 x 10 ±14.9 x 10, 21.5 x 10 ± 12.9 x 10 and 5.5 x 10 ± 3.5 x 10 cfu/gm raw, grilled and fired fish samples respectively with non significant difference between such means.

However, Morshidy and Hafez (1986), Hafez (1989), Naser (1991), Morshidy (1992a, b) recorded higher *Staph aureus* counts than the results of this investigation for raw fish where the mean figures were 4.8 x 10^2, 12.33 x 10^2, 1.3 x 10^3, 9.5 x 10^6 and 4.8 x 10^2 cfu/gm, respectively. Also, higher counts were recorded for fried fish by Adesiyun (1983) (2.6 x 10^6), Eldaly and Ibrahim (1987) (4.75 x 10^3) and Hefnawy (1990) (4 x 10^2) whereas the mean *Staph aureus* count /gm grilled fish was 6 x 10^2 cfu/gm as reported by Eldaly and Ibrahim (1987).

According to the results presented in Table 6,

it is evident that different microorganisms could be isolated in variant percentages from the examined 90 raw and ready-to- eat fish samples. These organisms were identified as *E.coli* (12.2%), *Staph. aureus* (21.1%), *C.perfrirgens* (25.6%), *Listeria* spp. (17.8%) of which *L.monocytogenes* constituted 6.7%, *Aeromonas* spp (47.8%), where the identified strains were *A.hydrophila* (23.3%), *A. caviae* (17.8%) and *A.Sorbia* (6.7%). On the other hand *Salmonella* organisms failed to be detected in any of the examined raw, grilled or fried fish samples.

Regarding *E.coli*., the findings illustrated in table 6 revealed that 13.3, 16.7 and 6-7 % of the examined raw, grilled and fried fish samples proved to harbour *E.coli*. However, Yousef *et al.* (1981), Yousef *et al.* (1985), Mahmoud (1999), El-Gohary and Samaha (1992), and Morshidy (1992 b) reported the isolation of *E.coli* from 7.92, 1.98, 6, 1.7 and 14% of raw fish while its recovery rate from fried fish was 12% as recorded by Hefnawy (1990).

It is clearly evident from the mentioned results in Table 6 that 30, 20 and 13.3% of the examined raw, grilled and fired fish samples contained *Staph aureus* Hefnawy, (1990) recorded that the incidence of *Staph aureus* was 20% in fired fish with was higher than that abtained in this study.

Small number of *Staph aureus* don't assure safety because it can produce enterotoxin and die during storage and processing but toxin remain in food (National Academy of Sciences, 1995).

As for *C.perfringens* in Table 6 it was existed in 46.7, 20 and 10% of the examined raw, grilled and fried fish samples respectively. Abd El-Rahman *et al.* (1989) were able to isolate *C.perfringens* from 10% of the examined raw fish samples.

However, Hefnawy (1990) could isolate the organism from 8% of fried fish whereas Moussa *et al.* (1992) reported an incidence of 26.6% in ready -to- eat fish. Besides, Rahmati *et al.* (2008) were able to isolate *C.perfringens* from 4.9% of raw and processed seafood.

C.perfringens spores can reach fish in their water habitat from surface of equipment and utensils used for processing and preparation or from workers, numbers greater than 10^6 are necessary to cause illness, (Bryan, 1980).

Listeria spp. were recovered from the examined raw, grilled and fried fish samples with an incidence of 26.7, 16.7 and 10%, respectively as shown in Table 6 *L.monocytogenes* was identified and constituted 10, 6.7 and 3.3% of the examined samples respectively.

The percentages of *Listeria* spp. in raw fish in this study was lower than that recorded by Ronda and Thaker (1992) (35%) and Ebrahim and Thabet (2007) (53%). On the other hand, the incidence of Listeria monocytogenes in the same product was nearly agreed with that results obtained by Mena *et al.* (2003) (12%), Ibrahim and Hassan (2006) (9.3%) and Wong *et al.* (1990) (10.5%) while Weagant *et al.* (1998) recoded 26% *L. monocytogenes* of greatest concern from public health point of view.

Dalton *et al.* (2004) found that the most frequently implicated vehicles in 17.3 out breaks were seafood and *L. monocytogenes* caused 40% of the deaths.

From the summarized results given in Table 6 it is evident that *Aeromonas* spp. Could be detected in 73.3% of the examined raw fish samples where the identified starins were *A.hydrophila* (40%), *A.caviae* (23.3%) and *A.sorbia* (10%).

On the other hand, 40 and 30% of the examined grilled and fried fish samples were positive for *Aeromonas* spp. The most prevalent strain was *A.hydrophila* (20 and 10%), followed by *A. caviae* (13.3 and 16.7%) and *A.sorbia* (6.7 and 3.3%).

However many investigators reported the isolation of different *Aeromonas* strains in variant percentages from raw and ready -to-eat fish examined as Gobat and Jainmi (1992) Henin (1995) Abd El-Daym (1999), Bastawrows and Mohamed (1999), Mahmoud (1999), Ammar (2001), Nasser (2005) and Hamdy *et al.* (2009).

Salmonellae failed to be recoverd from any of the examined fish samples either raw or

ready-to-eat fish, this results agreed the results obtained by Eldaly and Ibrahim (1987) and Hefnawy (1990) who couldn't isolate *salmonellae* from raw, or read to eat fish while Yousef *et al.* (1985) and Heinitiz *et al.* (1999), succeeded to isolate *salmonellae* from raw fish.

In conclusion, the present results revealed that fish may become contaminated with any of the foodborne pathogens where the level of contamination depends on the initial contamination and the opportunities for growth and/or survival processing and preparation of fish. Therefore, strict hygienic measures should be recommented to avoid contamination with these microorganisms: proper hand washing and disinfection, keeping raw and processed products, separated and implement handling and packaging practices that will limit the possibility of processed products becoming contaminated.

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