



## Detection of Aerobic Spore Formers in Ready to Eat Fish

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### ABSTRACT

A total of 300 fish samples were collected from Giza and Cairo shops, supermarkets and restaurants. these samples were 150 raw fish samples of Tilapia, Mackerel and Sardine (50 for each) and 150 ready to eat fish samples of fried Tilapia, grilled Mackerel and grilled Sardine (50 for each). samples were subjected to bacteriological examination for detection of aerobic spore forming bacteria. The obtained results indicated that, the infection rate with aerobic spore formers in raw Tilapia, Mackerel and Sardine were 60 %, 70 % and 100 %, respectively. While in fried Tilapia, grilled Mackerel and grilled Sardine were 30%, 14% and 20 %, respectively. The highest count of aerobic spore forming bacteria in raw fish samples found to be high in raw Sardine ( $7 \times 10^3$  cfu/gm) while the lowest one in raw Tilapia, Mackerel and Sardine were ( $1 \times 10^2$  cfu /gm) for all. In ready to eat samples the highest count found in fried Tilapia samples ( $6 \times 10^2$  cfu/gm). Four serotypes of aerobic spore formers recovered from raw fish; *B. cereus* , *B. subtilis*, *B. lanchiniformis* and *B. macerans* while in ready to eat fish the same serotypes were found besides *B. mycoides* .

**Keywords:** aerobic spore forming bacteria, fish, ready to eat fish.

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### 1. INTRODUCTION

Fish and fish products constitute an important food component for a large section of world population and in developing countries fish forms a cheap source of protein (Amusan et al., 2010). Food and Agriculture Organization (1994) asserted that fish contributes about 60% of the world's supply of protein and that 60% of the developing world derives more than 30% of their annual protein from fish. fish can transport many types of organisms from its natural aquatic environment, soil and

contaminated utensils during handling, processing distribution and storage (Shewan, 1971).

Ready to eat fish can also transmit microorganisms as cooking fish at very high temperature for short time kills all the vegetative bacteria except those that form heat-resistant spores, however, when the conditions become suitable, the growth rate of germinating spores would be high (Abd-El Rahman *et al.*, 2003). Aerobic spore forming bacteria which have epidemiological importance as some of their members are pathogenic and the results in

serious infections and food poisoning and the total numbers of these organisms can be taken as an indication of possible potential hazards to consumers (Borch *et al.*,1996).

*Bacillus* species, which represent one of aerobic spore formers are able to survive in certain heat treatments which applied during food processing (endospore resistance) and some psychrotrophic species can grow at low temperatures during food storage. Some species have been implicated in food spoilage such as *Bacillus licheniformis*, *Bacillus subtilis* and in food intoxication cases, in particular *B. cereus*, *B. licheniformis* and *B. subtilis* (Coton *et al.*, 2011). *Bacillus* spp. are extremely widespread in nature and may readily be isolated from soil, water, dust and air. The heat treatment of food was usually insufficient to kill thermophilic microorganisms and improper handling after cooking allowed such bacteria to multiply (Varnam and Evans, 1991). Therefore, this work was planned out to study the presence of aerobic spore forming bacteria in raw and ready to eat fish.

## 2. MATERIAL AND METHODS

### 2.1. Collection of the samples:

A total of 300 fish samples were collected from Giza and Cairo shops, supermarkets and restaurants. These samples were 150 raw fish samples of Tilapia, Mackerel and Sardine (50 for each) and 150 ready to eat fish samples of fried Tilapia, grilled Mackerel and grilled Sardine (50 for each). Each sample was kept in aspirate sterile plastic bag and put in an ice box then transferred to the laboratory under complete aseptic condition without delay. Sample

were subjected to bacteriological examination for detection of aerobic spore forming bacteria.

### 2.2. Preparation of the samples (APHA 1992):

Ten grams of fish muscle sample were aseptically transferred into a sterile blender flask containing 90 ml of sterile peptone water and homogenized for 2 minutes to provide a homogenate 1/10 dilution. However, one ml of the homogenate was transferred with sterile pipette to another tube containing 9ml of sterile peptone water, from which tenfold serial dilutions were prepared.

### 2.3. Determination of aerobic spore former count (according to Oxoid, 1990):

From each of the previously dilution 0.1 ml was evenly spread over the dry surface of duplicate plates of dextrose tryptone agar medium using a sterile glass spreader. The inoculated and control plates were incubated at 55°C for thermophilic bacteria and at 32°C for mesophilic bacteria for 48 hours. The aerobic spore formers count /g was calculated on plates containing 30-300 colonies and each count was recorded separately.

### 2.4. Identification of aerobic spore formers:

Suspected colonies of aerobic spore formers were picked up and sub cultured onto nutrient agar slopes then incubated at 37°C for 24 hours. The purified colonies were subjected for further identification according to (Kring and Holt ,1986) and (BAM ,1998).

### 3. RESULTS

Table (1) revealed that the incidence rate of aerobic spore formers in raw Tilapia, Mackerel and Sardine were 60 % (n=30), 70 % (n= 35) and 100 % (n=50), respectively. while in ready to eat fish the infection rate in fried Tilapia, grilled Mackerel and grilled Sardine were 30% (n=15), 14% (n=7) and 20 % (n=10), respectively.

Table (2) showed that the maximum aerobic spore forming bacterial count found to be the highest in raw Sardine ( $7 \times 10^3$  cfu/gm) while the lowest count in raw Tilapia, Mackerel and Sardine were ( $1 \times 10^2$  cfu /gm) for all. While in ready to eat fried Tilapia, grilled Mackerel, grilled Sardine the

lowest count were  $1 \times 10^1$ ,  $1 \times 10^2$  and  $1 \times 10^2$  and the highest count were  $6 \times 10^2$ ,  $5 \times 10^2$  and  $4 \times 10^2$  respectively.

Table (3) indicated that the serotypes of aerobic spore formers isolated from raw *Tilapia* fish were (*B. subtilis*, *B. cereus* and *B. lanchiniformis*) from raw Mackerel were (*B. subtilis* and *B. cereus*) and from raw sardines were (*B. subtilis*, *B. cereus*, *B. lanchiniformis* and *B. macerans*) While in fried Tilapia, the species which recovered were (*B. cereus* and *B. mycoides*), in grilled Mackerel (*B. subtilis*, *B. cereus* and *B. macerans*) and in grilled Sardine (*B. subtilis*, *B. cereus*, *B. lanchiniformis* and *B. macerans*).

Table (1): Incidence of aerobic spore formers in raw and ready to eat fish samples (n= 50):

Positive samples	Raw Tilapia	Raw Mackerel	Raw Sardine	P-value	Fried Tilapia	Grilled Mackerel	Grilled Sardine	P-value
Number	30	35	50	0.000*	15	7	10	0.170
percentage	60 %	70 %	100 %		30%	14%	20%	

\* By Chi square,  $P < 0.05$  ( $P = 0.000$ ); aerobic spore formers infection is more related to Sardine.

Table (2): analytical results of total aerobic spore formers count cfu / gm in raw and ready to eat fish samples (n=50):

Count	Raw Tilapia	Raw Mackerel	Raw Sardine	Fried Tilapia	Grilled Mackerel	Grilled Sardine
Minimum	$1 \times 10^2$	$1 \times 10^2$	$1 \times 10^2$	$1 \times 10^1$	$1 \times 10^2$	$1 \times 10^2$
Maximum	$2 \times 10^3$	$3 \times 10^2$	$7 \times 10^3$	$6 \times 10^2$	$5 \times 10^2$	$4 \times 10^2$

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Table (3): serotypes of aerobic spore formers isolated from raw and ready to eat fish samples (n=50):

Bacterial isolates	Raw Tilapia	Raw Mackerel	Raw Sardine	Fried Tilapia	Grilled Mackerel	Grilled Sardine
Number	30	35	50	15	7	10
Type of aerobic spore formers	<i>B. lanchniformis</i> <i>B. cereus</i> <i>B. subtilis</i>	<i>B. cereus</i> <i>B. subtilis</i>	<i>B. macerans</i> <i>B. lanchniformis</i> <i>B. cereus</i> <i>B. subtilis</i>	<i>B. mycooides</i> <i>B. cereus</i>	<i>B. macerans</i> <i>B. cereus</i> <i>B. subtilis</i>	<i>B. lanchniformis</i> <i>B. macerans</i> <i>B. cereus</i> <i>B. subtilis</i>

### 4. DISCUSSION

Fish is very important for human nutrition because it is rich in animal protein and other elements for the maintenance of healthy body (Andrew, 2001). Raw or cooked fish may be harbored aerobic spore forming bacteria which can cause human illness after consuming.

The results given in table (1) much higher than result detected by (Goja ,2013) in Sudan where the percentage of raw Tilapia spp. infection with Bacillus spp. was 10.1 %. The high results in this study may be attributed to fish freezing in the seawater, intensive handling, long-time transport, cross contamination from environment, source and handling by the sellers, contamination of fish container with microorganisms, poor hygienic practice which increase the contamination of fish due to unsanitary procedures, the rotation of the assigned duties of workers and airborne microorganisms (Novotny et al., 2004). The high bacterial load in raw Mackerel and raw Sardine may be due to the oily nature of such fishes in which rancidity as a result of

fat oxidation lead to spoilage by a great number of microorganisms (Ayuba et al., 2013 and Ababouch et al., 1996). Also these results were agreed with (Chukwu et al., 2013) who detected Bacillus spp. in a percentage of 16% in ready-to-eat fried samples of hawked fish (*Trachurus capensis*) in Nigeria. The result was different with (Hassanien et al., 2014) who could not isolate aerobic spore formers from fried seafood (*Mugil cephalus*, *Saurus*, *Sepia*, *Shrimp*).

In table (2) the result agrees with (El-Dengawy et al., 2012) who mentioned that the count of aerobic spore formers in 2 samples of frozen mackerel were  $0.044 \pm 0.044 \times 10^3$  and  $1.30 \pm 0.760 \times 10^3$  cfu /gm respectively. Also, the results was agreed with (Mansour, 2001) who found that in one hundred and twenty random samples of smoked fish, salted sardines, canned salmon and canned tuna the total aerobic spore formers were ranged between  $1 \times 10^2$  to  $5 \times 10^1$ ,  $1 \times 10^1$  to  $3 \times 10^1$ ,  $1 \times 10^1$  to  $5 \times 10^1$  and  $1 \times 10^1$  to  $3 \times 10^1$  cfu/gm, respectively.

The variation in the count of aerobic spore formers in ready to eat fish may be

due to the variation in post-cooking contamination, unsatisfactory hygienic measures in handling and processing of fish, destroying the bacteria by proper cooking with high temperature and contamination during packaging and processing (Nickelson and Finne, 1984).

Result in table (3) agree with these reported by (El-Shamery, 2010) in Yemen who recorded *B. subtilis*, *B. pumilus*, *B. circulans*, *B. lentus*, *B. macerans* and *B. cereus* in local fresh fish meats (fish common name is Gahsh), and was agreed with (Aruwa and Akinyosoye, 2015) who detected *Bacillus cereus*, *Bacillus subtilis*, *B. megaterium*, *B. thuringiensis*, *B. licheniformis* and *B. mycoides* in some ready-to-eat foods (Buns, Meat pie, Egg roll, White rice, Jollof rice and Fried rice).

While the current results were disagreed with (El-olemy *et al.*, 2014) who could not isolate *Bacillus* spp. between the zoonotic bacteria that they isolated from some market fish (*Oreochromis niloticus* and *Clarias gariepinus*) in Qalyoubia province.

In this study it was concluded that the infection of raw fish and ready to eat fish can be infected by aerobic spore forming bacteria and the incidence of these microbes in raw fish was higher than in ready to eat fish specially in raw Mackerel and Sardine (70 % and 100 %) in spite of that the cooking process may decrease but not kill the bacteria due to spore formation which resist the harsh environmental condition as high temp. so it is important to make strict hygienic measures in handling and preparation of fish from fishing to

consumption to avoid contamination with aerobic spore formers.

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