Assessment of Psychrotrophic Bacteria in frozen fish with special reference to Pseudomonas Species

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A B S T R A C T

Frozen fish exposed to many risks of contamination during long chain of catching, transportation, dressing and freezing from different sources till they reach to consumers which make it harmful or unfit for human consumption.

A total of 150 random samples of frozen fish of Mackerel, Saurus, *Mugil Cephalus, Horse Mackerel* and Sardine (30 of each) were collected from different fish markets at Kaliobia Governorate for determination of Psychrotrophic, Pseudomonas and Aeromonas count in frozen fish samples. The mean value of Psychrotrophic , Pseudomonas and Aeromonas count (cfu/g) were $5.11x10^5$ $\pm 2.4x10^5$, $9.92X10^4$ $\pm$ 4.8 x $10^4$and 4.8 x 10^4 $\pm$ 1.17 x 10^4 in Mackerel, $1.18x10^5$ $\pm 2.9x10^4$, 1.76 X 10^5 $\pm$ 6.16 x 10^4 and 4.78 x 10^4 $\pm$ 2.07 x 10^4 in Saurus, $8.90x10^4$ $\pm 3.4x10^4$, 6.35x 10^4 $\pm$ 3.9 x 10^4 and 9.18 x 10^2 $\pm$ 2.6 x 10^2 in Mugil Cephalus, $2.67x10^4$ $\pm 1.26x10^4$, 9.35 x 10^2 $\pm$ 2.08 x 10^2 and 7.96 x 10^2 $\pm$ 1.73 x 10^2 in Horse Mackerel, $3.58x10^4$ $\pm 1.17x10^4$,6.87x 10^2 $\pm$ 1.14 x 10^2 and 6.17x 10^2 $\pm$ 1.35 x 10^2 in Sardine, respectively. There were observed significant difference ($P<0.05$) between examined samples. Moreover ,the result obtained showed that Mackerel is the most contaminated fish with Psychrotrophic and Aeromonas bacteria. However, Saurus is the most contaminated fish with *Pseudomonas* followed by Mackerel , *Mugil Cephalus, Horse Mackerel* and finally Sardine. From the obtained results it could be concluded that the most prevalence *Psychrotrophic bacteria* isolated from frozen fish samples were *Pseudomonas, Aeromonas* followed by *Achromobacter* and these bacteria consider a major factor for the spoilage of fish or be a health hazard.

Keywords: frozen fish, Psychrotrophs, Pseudomonas, Aeromonas, Mackerel, Saurus, *Mugil Cephalus, Horse Mackerel,* Sardine.

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1. INTRODUCTION

Fish consider the main source of high quality protein that we can easily and completely digest.it is very rich source of vitamins as vit B6 , B12 and rich in mineral as iodine , Ca, Ph that are vital to our health in addition fishes are excellent source of un saturated fatty acid which protect fish eater against heart diseases, obesity and hypertension.

Freezing not a method of sterilization but it makes fish keep its flavor, odor, texture for

long time. It also reduces deterioration caused by microorganism, enzyme, chemicals as O2. Many of the associated microbial cells are killed or damaged by freezing(Mahmoud,1994).The presence of Psychrotrophic organisms may be attributed to environmental contamination around the fish , The processing operation, equipments, workers, container, boxes and work services establishments, as well as, using polluted water during transportation play an important role to increase the bacterial load of Psychrotrophic bacteria, delaying refrigeration after harvesting and other handling errors between harvesting and processing lead to decomposition of the sea fish and allow microbes to grow rapidly,(Marriott, 1997).

Psychrotrophsare these bacteria that grow well at or below 7℃ and have their optimum temperature for growth between 20-30℃. Some Psychrotrophic pathogens can grow in the refrigerated food with little or no obvious change of sensory characteristics (Berrang et al.1989).

Generally, Psychrotrophic bacteria may cause harmful health condition such as sepsis, diarrhea, meningitis, dysentery, food poisoning, urinary tract Infections and gastrointestinal infections .According to studies, people who have greater risks of getting exposed to these harmful bacteria are those who are pregnant, newborns, immune-compromised and have pre-existing health. Thus, their presence in food creates a great risk as they lead to food poisoning and spoilage of food (Jay, 2000).

Microbial spoilage of fish usually is described as a proteolytic process, Pseudomonas spp.is considered the most important Psychrotrophic microorganisms causing fish spoilage and therefore, illness results from eating of such fish or its products may occur, although signs of spoilage may not be evident(Amany,2004).Pseudomonas species are widely distributed in soil and water. They commonly recognized as human and animal pathogens and other species may cause food spoilage (Carter et al., 1990). Accordingly, Pseudomonas species are important spoilage organisms in many chilled food products especially fish, in which they become the dominant microflora during chill storage(Gram,1993).In addition, their presence in fish create a great risk as they lead to poisoning and/or spoilage of fish(Jay,2000).

Aeromonads cause septicemia and gastroenteritis in human (Ko et al., 2000). Also, they causes extra intestinal infections involved septicemia, hemolytic uremic syndrome, meningitis, peritonitis, wound infection and respiratory infection (Janda and Abbot, 1998 and FDA, 2001).Therefore, the study was planned out to assessment of prevalence of Psychrotrophic, Pseudomonas and Aeromonas count in some frozen fish (Mackerel, Saurus, Mugil Cephalus, Horse Mackerel, Sardine) and Isolation as well as identification of Pseudomonas species from such examined samples.

2. Materials and methods

2.1. Collection of samples:

A total of 150 random samples of frozen fish of Mackerel, Saurus, Mugil Cephalus, *Horse Mackerel* and Sardine (30 of each) were collected from different fish markets at Kaliobia Governorate. All collected samples were transferred directly to the laboratory under complete aseptic condition without undue delay and thawed at refrigerator until subjected to bacteriological examinations.

2.2. Bacteriological examinations:

Preparation of samples (AOAC, 1990):

Fish samples were based on its side over sterile plate hold by sterile forceps. The body surface was sterilized by ethyl alcohol and

flame, and then the sterilized surface was removed by sterile forceps. Under complete aseptic condition, 10 gm of the back muscle were transferred into sterile homogenizer jar containing 90 ml of sterile 0.1% peptone water. The contents were homogenized for 2.5minutes at room temperature(20℃) and then allowed to stand for 5 minutes. One ml of homogenate was transferred into sterile test tube contained 9ml of 0.1% peptone water 10^{th} fold serial dilution were prepared up to10^{6} .

-Determination of Psychrotrophic count: according to (Collins and Lynel, 984):

-Determination of Pseudomonas count: according to (ICMSF, 1978):

-Isolation and identification of Pseudomonas species:

The purified colonies were subjected for further identification either morphological and Biochemical according to Krieg and Holt (1984).

-Determination of Aeromonas count: according to (APHA, 1992).

-Statistical Analysis: ANOVA test according to (Feldman et al., 2003).

RESULTS

As shown in table (1) the result revealed that the mean value of Psychrotrophic count (cfu/ g) were $5.11x10^{5}$ $\pm2.4x10^{5}$in Mackerel, $1.18x10^{5}$ $\pm2.9x10^{4}$in Saurus,$8.90x10^{4}$ $\pm3.4x10^{4}$ in *Mugil Cephalus*, $2.67x10^{4}$ $\pm1.26x10^{4}$ in *Horse Mackerel* and $3.58x10^{4}$ $\pm1.17x10^{4}$ in Sardine, respectively. Significant differences ($P<0.05$) were observed between different frozen fish samples. Moreover, serovars identification of *Psychrotrophic bacteria* showed in table (2)were serologically identified as Pseudomonas , Aeromonas, Achromobacter, Serratia (gram – ve bacteria) in percentages of 15(50%), 6(20%), 6(30%), 3(10%) in Mackerel, 12(40%), 3(10%), 3(10%), 6(20%) in Saurus., 18(60%), 9(30%), 3(10%), 3(10%) in Mugil Cephalus., 9(30%) , 6(20%) , 3(10%) ,3(10%) in *Horse Mackerel*, and 15(50%), 9(30%), 9(10%), 0 in Sardine, respectively. And identified gram positive Psychrotrophic bacteria were Bacillius spp., Micrococci, Staphylococci in a percentages of 3(10%) , 3(10%) , 9(30%) in Mackerel, 3(10%), 0, 0 in Saurus , 6(20%) , 9(30%), 6(20%) in *Mugil Cephaus*, 3(10%), 3(10%) , 3(10%) in *Horse Mackerel* and12(40%), 6(20%), 3(10%) in Sardine,. respectively.

Presence of Pseudomonas in examined samples lead to color defect in fish fillets, some species have been suggested as causative agents of food borne illness, *Pseudomonas aeruginosa* produce enterotoxin cause gastroenteritis. The Psychrotrophic Pseudomonades are found in all types of refrigerated and frozen food, (George and Banwart, 1979).The genus of Pseudomonas comprises more than 140 species, but only one of those species was *Pseudomonas aeruginosa* which is pathogenic to man particularly in newborn babies, as well as urinary tract infections, burns and eye infections. The infections with Pseudomonas Species become generalized in immunosuppressive peoples (Visatil et al., 1998).

the results recorded in(table 3)showed that the mean values of Pseudomonas counts(cfu/g) were $9.92X10^{4}$ $\pm4.8 \times 10^{4}$, $1.76X\ 10^{5} \pm 6.16 \times 10^{4}$, $6.35x\ 10^{4} \pm 3.9 \times 10^{4}$, $9.35x\ 10^{2} \pm 2.08 \times 10^{2}$,and $6.87x\ 10^{2} \pm 1.14 \times 10^{2}$ in Mackerel, Saurus, *Mugil Cephalus*, Horse Mackerel and Sardine, respectively.it also decleared that, the mean values of Aeromonas count (cfu/g)in Mackerel, Saurus, *Mugil Cephalus*, *Horse Mackerel* and Sardine were$4.8x10^{4}\pm1.17x10^{4}$,$4.78x10^{4}\pm2.07x10^{4}$, $9.18x10^{2}\pm2.6x10^{2}$, $7.96x10^{2}\pm1.73x10^{2}$ and $6.17x10^{2}\pm1.35x10^{2}$, respectively.

Moreover, serovars identification of *Pseudomonas* species showed in table (4)

were serologically identified as, *Ps. Aeruginosa, Ps. Fluorescence, Ps. alcaligenes, Ps. diminatus, Ps .putida, Ps .fragi and Ps. Stiutzeri* in incidence of 3 (10%), 12 (40%), 3(10%), 3 (10%), 3 (10 %), 3(10%), 3 (10%) in Mackerel, 3 (10%), 18 (60%), 0 , 0, 6 (20%) , 3 (10 %), 0inSaurus, 3(10%), 18 (60%), 0 , 0, 6(20%), 3(10%), 0 in *Mugil Cephalus*., 0, 12(40%) , 6(20%) , 6(20%) , 6(20%), 0,0 in *Horse Mackerel* and 3(10%), 12(40%), 6(20%), 3(10%) , 6(20%),0,0 in Sardine, respectively.

Table 1: Mean values of Psychrotrophic fu/g in the examined samples of frozen fish (n=30).

Type of Fish	Min.	Max.	Mean±SE*
Mackerel	$1.8x10^3$	$4.5x10^6$	$5.11x10^5 \pm 2.4x10^5$
Saurus	$1.1x10^3$	$3.6x10^5$	$1.18x10^5 \pm 2.9x10^4$
Mugil Cephalus	$1.1x10^2$	$6.1x10^5$	$8.90x10^4 \pm 3.4x10^4$
Horse Mackerel	$1.4x10^2$	$2.3x10^5$	$2.67x10^4 \pm 1.26x10^4$
Sardin	5.4x10	$1.8x10^5$	$3.58x10^4 \pm 1.17x10^4$

SE*=stander error of mean

Table 2: Serotypes of *Psychrotrophic* bacteria isolated from the fish samples (n=30).

Microorganism	Mackerel		Saurus		Mugil cephalus		Horse Mackerel		Sardine	
	No.	%	No.	%	No.	%	No.	%	No.	%
Grame –ve bacteria:										
Pseudomonas	15	50	12	40	18	60	9	30	15	50
Aeromonas	6	20	3	10	9	30	6	20	9	30
Achromobacter	9	30	3	10	3	10	3	10	3	10
Serratia	3	10	6	20	3	10	3	10	0	0
Grame +ve bacteria:										
Bacillus spp.	3	10	3	10	6	20	3	10	12	40
Micrococci	3	10	0	0	9	30	3	10	6	20
Staphylococci	9	30	0	0	6	20	3	10	3	10

No= number of isolates.

Table 3: Mean values of Pseudomonas, Aeromonas (cfu/g) in frozen fish samples (n=30).

Type of Fish	*Pseudomonas* +ve samples			*Aeromonas* +ve samples		
	No	%	Mean±SE*	No	%	Mean±SE*
Mackerel	18	60%	$9.92x10^4$±$9.02x10^4$	15	50%	$4.8x10^4$±$2.19x10^4$
Saurus	18	60%	$1.7x10^5$±$1.13 x 10^4$	24	80%	$4.78x10^4$±$3.7x10^4$
Mugil cephalus	21	70%	$2.2x10^3$±$9.05x10^2$	18	60%	$9.19x10^2$±$4.91x10^2$
Horse Mackerel	21	70%	$9.3x10^2$±$3.8 x10^2$	15	50%	$7.96x10^2$±$3.25x10^2$
Sardin	21	70%	$6.8x10^2$±$2.08x10^2$	15	50%	$6.17x10^2$±$2.53x10^2$

SE*=stander error of mean

Table 4: Serotyping of Pseudomonas species isolated from the frozen fish samples (n=30).

Microorganism	Mackerel		Saurus		*Mugil cephalus*		*Horse Mackerel*		Sardin	
	No.	%	No.	%	No.	%	No.	%	No.	%
Ps. aeruginosa	3	10	3	10	3	10	0	0	3	10
Ps. fluorescence	12	40	18	60	18	60	12	40	12	40
Ps alcaligenes	9	10	0	0	0	0	6	20	6	20
Ps, diminata	3	10	0	0	0	0	6	20	3	10
Ps. putida	3	10	6	20	6	20	6	20	6	20
Ps. fragi	3	10	3	10	3	10	0	0	0	0
Ps. stuteori	3	10	0	0	0	0	0	0	0	0

4. DISCUSSION

Growth of Psychrotrophic bacteria in fish has become a significant problem due to the wide spread use of refrigerated storage of fish and in processing factories. Psychrotrophic bacteria when grow in fish can induce different varieties of off- flavor including fruity, stale, bitter, putrid, rancid flour as well as other physical defects.

It is evident from the result recorded in table(1) that the highest contaminated frozen fish samples were *Mackerel* because it contains high amount of fat and oils followed by Saurus, *Mugil cephalus* and the

lowest ones were Sardine and finally *Horse Mackerel*.

These results came in a accordance with those reported by El-Shafey(2014) (4.08 x 10^5 ± 0.71 x 10^5cfu / g in *Saurus*, 9.95 x 10^4 ±2.13 x10^5 cfu/g in *Mackerel* and3.66 x 10^4 ± 0.49 x 10^4in *Horse Mackerel* ,respectively) and Elsayed (2016) found that the mean value of Psychrotrophic count (cfu/g) in frozen *Mugil Cephalus* was 1.72 x 10^5 ± 0.46 x 10^5 . Lower results of Psychrotrophic count (cfu/g) were recorded by Nermeen (2006)she found that the mean values of Psychrotrophic *c*ount in imported Mackerel and Sardine were 4.6 ×10^3 ± 1.2×10^2cfu/g and 3.7×10^3 ± 1.3×10^2cfu/g. Higher results of Psychrotrophic count were obtained by El-Noby ,(2002) (1.0 ×10^7 ± 2.7×10^6cfu/g and 2.4 ×10^{10} ± 8.2×10^6cfu/gin frozen *Mugile Cephalus* and Mackerel).

The most gram +ve Psychrotrophic bacteria contaminated these examined samples were *Pseudomonas species* followed by Aeromonas and Achromobacter, Serratia not be isolated from Sardine (table 2). Nearly similar results were obtained by Elsayed (2016) who isolated Pseudomonas and Aeromonas species from frozen *Mugil Cephalus*9(60%), 6(40%) . lower results were obtained by Abou EL-Atta(2003)who isolated *Ps.spp*. in a percentage of 26.05%. Higher results were obtained by ELshafey (2014) that isolate Pseudomonas and Aeromonas species from frozen Saurus 23(76.67%), 19(63%), Mackerel 20(66.67%), 16(53.33%), and *Horse Mackerel* 19(63.33%) ,14(46.67%) respectively. The most gram –ve Psychrotrophic bacteria contaminated these examined samples were *Bacillius spp*. followed by Micrococci, and Staphylococci. In contrast Micrococci and Staphylococci were not isolated from Saurus.

Presence of Pseudomonas in examined samples lead to color defect in fish fillets, some species have been suggested as causative agents of food borne illness, *Pseudomonas aeruginosa* produce enterotoxin cause gastroenteritis. The Psychrotrophic Pseudomonades are found in all types of refrigerated and frozen food, (George and Banwart, 1979).The genus of *Pseudomonas* comprises more than 140 species, but only one of those species was *Pseudomonas aeruginosa* which is pathogenic to man particularly in newborn babies, as well as urinary tract infections, burns and eye infections. The infections with Pseudomonas Species become generalized in immunosuppressive peoples (Visatil et al., 1998).

The result archived in (Table 3) revealed that the highest contaminated fish with Pseudomonas counts (cfu/g) was Saurus followed by Mackerel and Mugil *Cephalus* and the lowest one was *Horse Mackerel* and finally Sardine. The variation in the result between different species may be due to the effectiveness of hygienic measures applied during catching, freezing, storage and thawing method. The archived results nearly similar to ELshafey (2014) who found that Saurus was the most contaminant fish followed by Mackerel and *Horse Mackerel* finally.

In human, Aeromonas species including *A. hydrophila, A. caviae and A .veronii biotype sobria* caused gastrointestinal and extra -intestinal infections. Specially, *A.hydrophila* causes septicemia, hemolytic uremic syndrome, meningitis, peritonitis, wounds and respiratory diseases (Janda and Abbott, 1998).So, it is of great importance to mention that ES (2005) stipulated that the fish should be free from food pathogen therefore, the presence of *A. hydrophila* constitute a public health hazard.

Moreover the result obtained for Aeromonas showed that the examined Mackerel samples were more contaminated than other samples followed by Saurus, *Mugil Cephaus , Horse Mackerel* and the lower one was Sardine and this may be attributed to the nature of structure of Mackerel as it contains high percentage of fat and oil in their meat which act as good media for growth and multiplication of microorganisms.

As shown in (table 4) results illustrated that *Ps. alcaligenes* and *Ps. Diminatus*, were not isolated from *Saurus* and *Mugil Cephalus*, Ps. aeruginosa in Horse *Mackerel, Ps.fragi* in *Horse Mackerel and Sardine* and *Ps.stiutzeri* in *Saurus, Mugil Cephalus, Horse Mackerel* and *Sardine*. In contrast, most examined samples of frozen fish were highly contaminated by *Ps. fluorescens*, followed by *Ps .putida, Ps. alicaligenes*.

Nearly similar percentages were recorded by, ELshafey (2014) that *Ps. Fluorescens* was recovered in a rate of 16 (53.33%), 14(46.67%), 11(36.67%) from Saurus, *Mackerel, Horse Mackerel*, respectively, Elsayed (2016) found that *ps .fluorescens* was isolated in a rate of 9(60%) from *Mugil Cephalus*. Lower results were obtained by Amany (1997) who isolated *Ps. Fluorescens biovar II* in a rate of 33.3% and 32.1%, from *Mugil Cephalus and* Mackerel, Iman (2004) who revealed that incidence of *Ps. flouresens* (36.9%), *Ps. alcaligenes* (15.5%) .

5. **Conclusion**

The most prevalence *Psychrotrophic bacteria* isolated from frozen fish samples were *Pseudomonas, Aeromonas followed* by *Achromo bacter* and these bacteria consider a major factor for the spoilage of fish or be a health hazard. the highest contaminated frozen fish samples with Psychrotrophic bacteria, Pseudomonas and Aeromonas were Mackerel and Saurus followed by *Mugil Cephalus* and the lowest ones were Sardine and Horse Mackerel. This contamination of fish may be attributed to many factors make fish loss its quality and cause public health hazard so good hygienic measures should be applied during catching, transportation, manufacturing, freezing chamber and marketing of frozen fish.

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