PSYCHROTROPHIC BACTERIA IN FROZEN FISH WITH SPECIAL REFERENCE TO PSEUDOMONAS SPECIES

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A B S T R A C T

A total of 90 random samples of Saurus, Mackerel and Horse Mackerel (30 of each) were collected from different fish markets in El- Kalubia governorate to be examined bacteriologically for determination of psychrotrophic and *Pseudomonas* count in such examined samples. The bacteriological examination revealed the mean values of total psychrotrophic count and *pseudomonas* count in the examined samples of Saurus were 4.08 x 10^5 ± 0.71 x 105and 1.26 x 10^4 ± 0.32 x 10^4 cfu/g respectively. 9.95 x 10^4 ± 2.13 x 10^4, and 5.13 x 10^3 ± 0.91 x 10^3 x 10^3/ gm respectively. in Mackerel ,3,66 x 10^4 ± 0.49 x 10^4, and 2,47 x 10^3 ± 0.55 x 10^3 /g, in Horse Mackerel.. The incidence of Pseudomonas species were 76.67% 66.67% and 63.33% of the examined samples of Saurus, Mackerel and Horse Mackerel, respectively. The differences between the examined samples of different frozen fish as result of total psychrotrophic count, and total pseudomonas count were highly significant (P< 0.01). Public Health significant of isolated psychrotrophic bacteria and possible sources of fish contamination with such organisms as well as some recommendations to improve the quality of fish were discussed.

Keywords::Fish Saurus, Mackerel, Horse Mackerel, Psychrotrophs, Pseudomonas

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1. INTRODUCTION

Fish has long been regarded as a nutrition and highly desirable food due to its contribution of high quality animal protein, richness in calcium and phosphorus and generous supply of vitamins. Freezing of fish has the advantage of providing the consumer with unprocessed fish that retain to a much greater extent the flavor, odor, appearance and texture of the freshly caught fish. However, freezing is not a method of sterilization; it is assumed that the process of catching, dressing, transporting, freezing and thawing may expose the frozen fish to many risks of contamination from different sources. Such contamination may render the product unfit for human consumption or even harmful to consumers (Halda – Alija, and Subangi, (2004). Psychrotrophs are thesebacteria that grow well at or below 7°C and have their optimum temperature for growth between 20-30°C. Some psychrotrophic pathogens can grow in the refrigerated food with little or no obvious change of sensory characteristics (Berrong *et al.,* 1989). Pseudomonas species are important spoilage microorganisms in many chilled food products especially fish in which they become the dominant microflora during chill storage (Gram, 1993). Thus, their presence in food creates a great risk as they lead to food poisoning and spoilage of food (Jay, 2000). Therefore, this work was planned out to determine the psychrotrophic and pseudomonas count in frozen fish (Saurus mackerel, Horse mackerel) and isolation as well as identification of Pseudomonas species from such examined samples.

2. MATERIAL AND METHODS

2.1. Collection a fish samples:

A total of 90 random samples of Saurus, Mackerel and Horse mackerel (30 of each) were collected from different fish markets at Kalyobia governorate. All collected samples were examined bacteriologically as rapidly as possible for determination of total psychrotrophic, and Pseudomonas counts.

2.2. Preparation of the samples (APHA, 1984):

Accurately 25 grams of examined fish flesh were transferred to a sterile polyethylene bag and *225* ml of 0.1% sterile buffered peptone water in a blender at 2000 rpm for 1-2 minutes to provide a homogenate of 1/10 dilution. One ml from the original dilution was transferred with sterile pipette to another sterile test tube containing 9ml of sterile buffered peptone water (0.1%) and mixed well to make the next dilution, from which further decimal serial dilutions were prepared. The prepared dilutions were subjected to the following examinations:

2.2.1. Determination of Psychrotrophic count (Collins and Lyne1984):

From each dilution, 1 ml of the homogenate was transferred by using a sterile pipette into two separate sterile Petri-dishes to which approximately 15 ml of sterile melted and tempered plate count agar (45°C) were added and mixed. The inoculated plates were gently shaken in rotatory movement and left till complete solidification of the agar. The plates were inverted and incubated at 7°C for 10 days. The total psychrotrophic count/g was calculated on plates containing 30-300 colonies.

2.2.2.2 Determination of total Pseudomonas count (ICMSF, 1978):

Accurately, 0.1 ml. homogenate were separately inoculated into duplicate Petri-dishes of Pseudomonas selective agar medium supplemented with glycerol and evenly spread. The inoculated plates were incubated at 25 °C for 48 hours after which all developed colonies (greenish yellow colonies) were enumerated. The average count was calculated and recorded.

The suspected colonies were purified and subcultured onto nutrient agar slopes and incubated at 37°C for 24 hrs. The purified colonies were subjected for further identification either morphologically (Gram stain, Motility test or biochemically according to Kreig and Holt (1984).

3. RESULTS

Regarding the results recorded in table (1), it is obvious that the total psychrotrophic count/ cfu/g of the examined samples of frozen fish varied from $6.3x10^4$ to $5.7\ x10^6$ with an average of $4,0^8x10^5 \pm 0.71\ x\ 10^5$ for Saurus, $1.1x10^4$ to $2.4\ x\ 10^6$ with an average of $9.95\ x\ 10^4 \pm 2.13\ x\ 10^4$ for Mackerel and $8.2\ x\ 10^3$ to $6.9\ x\ 10^5$ with an average of $3.66\ x\ 10^4 \pm 0,48\ x\ 10^4$ for Horse mackerel, respectively. Incidence of identified psychrotrophic bacteria isolated from the examined samples of frozen fish was shown in table (2). Accurately , *Acinebacter, Alcaligenes, Chromobacterium, Flavobacterium, Moraxella* and *Neisseria* were isolated from the examined samples of frozen fish The most psychrotrophic bacteria contaminated the examined samples of frozen fish was Alcaligenes which isolated with high incidence (33.33%, 23.33% and 30%) followed by Flavobacterium (30% , 13.33% and 6.67%) in Saurus, Mackerel and Horse mackerel, respectively. Results given in table (3) revealed that the total Pseudomonas counts (cgu/g) in the examined samples of frozen fish ranged from $1.5x10^3$ to $4.4\ x\ 10^4$ with a mean value of $1.26X104 \pm 0.32\ x\ 10^4$ for Saurus, $7.0\ x\ 10^2$ to $1.1\ x\ 10^4$ with a mean value of $5.18X\ 10^3 \pm 0.91\ x\ 10^3$ for Mackerel and $3.4\ 10^2$ to $5.9\ x\ 10^3$ with a mean value of $2.47x\ 10^3 \pm 0.55\ x\ 10^3$ for Horse mackerel, respectively. Incidence of identified of *Pseudomonas spp.* isolated from the examined samples of frozen fish

Table 1. Statistical analytical results of total psychrotrophic counts in the examined samples of frozen fish (n=30).

Type of fish	Min.	Max.	Mean ± S.E*.
Saurus	6.3×10^4	5.7×10^6	$4.08\times10^5\pm 0.71\times10^{5++}$
Mackerel	1.1×10^4	2.4×10^6	$9.95\times10^4\pm 2.13\times10^5$
Horse Mackerel	8.2×10^3	6.9×10^5	$3.66\times10^4\pm 0.49\times10^4$

S.E* = standard error of mean
++ = High significant differences

Table 2. Incidence of identified psychrotrophic bacteria isolatedfrom the examined samples of frozen fish (n=30).

Fishtype	Saurus		Mackerel		Horse Mackerel	
Psychrotrophic bacteria	No.	%	No.	%	No.	%
Acinetobacter	2	6.67	5	16.67	1	3.33
Alcaligenes	10	33.33	7	23.33	9	30.00
Chromobacterium	4	13.33	2	6.67	6	20.00
Flavobacterium	9	30.00	4	13.33	2	6.67
Moraxella	7	23.33	4	13.33	5	16.67
Neisseria	3	10	1	3.33	1	3.33

* The percentages were calculated according to total number of samples

Table 3. Statistical analytical results of total *Pseudomonas* counts in the examined samples of frozen fish (n=30).

Type of fish	Min.	Max.	Mean ± S.E*.
Saurus	1.5×10^3	4.4×10^4	$1.26\times10^4\pm 0.32\times10^{3++}$
Mackerel	7.0×10^2	1.1×10^4	$5.18\times10^3\pm 0.91\times10^3$
Horse Mackerel	3.4×10^2	5.9×10^3	$2.47\times10^3\pm 0.55\times10^3$

S.E* = standard error of mean
++ = High significant differences

Table 4. Incidence of identified Pseudomonas species isolated from the examined samples of frozen fish (n=30).

Fishtype	Saurus		Mackerel		Horse Mackerel	
Pseudomonas species	No.	%	No.	%	No.	%
Ps. acidovorans	3	10.00	1	3.33	-	-
Ps. alcaligenes	8	26.67	10	33.33	7	23.33
Ps. diminuta	5	16.67	2	6.67	3	10.00
Ps. fluorescens	16	53.33	14	46.67	11	36.67
Ps. multophila	2	6.67	-	-	-	-
Ps. putida	9	30.00	6	20	4	13.33
Ps. putrefaciens	11	36.67	5	16.67	8	26.67
Ps. stutzeri	1	3.33	-	-	-	-
Ps. vesicularis	5	16.67	7	23.33	2	6.67

* The percentages were calculated according to total number of samples

were shown in table (4). Actually, *Ps. acidovorons. Ps. alcaligenes, Ps. diminatus, Ps. fluorescence, Ps.* multophilla*, Ps.putida, Ps. putrefaciens, Ps.stiutzeri* and *Ps. vesicularis*

4. DISCUSSION

The psychrotrophic bacteria have received an increased attention by several investigators during recent years because the modern developments in fish and fish products have resulted in that fish must be held for long period at low temperature which greatly slow the multiplication of bacteria, but not stop their growth, providing favorable conditions for growth of psychrotrophic bacteria. Results which obtained in table (1) shown that the examined samples of Saurus were more contaminated with psychrotrophic bacteria than the examined samples of Mackerel and Horse mackerel because it containshigs amount of fats and oils. The present results come in accordance with those reported by Amany (2004), Gonzalez *et al.* (1999) and Hamza (2004) who reported that the average of psychrotrophic count were $3.84x10^4$ ±8.78 x 10 ^{3}/g respectively. The high psychrotrophic count of frozen fish may be attributed to the contamination of raw materials which come in contact with fish unsatisfactory sanitation during handling, processing and distribution as well as inadequate chilling and/or freezing which increase the existing microorganisms (Thatcher and Clark, 1978). Psychrotrophic bacteria when grow in fish can induce different varieties of off flavor including fruity, stale, bitter, putrid, rancid flavor as well as other physical defects (Jay, 2000). Table (3) indicated that the examined samples of saurus were more contaminated with *seudomonas* species than the examined samples shown that Saurus is higher contaminated with *Pseudomonas* species than Mackerel and Horse mackerel this could be attributed to the saurus fish contatain more oils than other species of fish. The obtained results were nearly similar to the results which obtained by El-Noby (2002), Amany (2004) and Mansour *et al.* (2009) who recorded that *Pseudomonas* count varied from $1.2 x10^3$ to $2.3x10^5$ with an average of $5.7 x 10^4$ ±3.5 x 10^3 for Tilapia, $1.8 x 10^2$ to $3.8 x10^4$with an average of $1.4 x 10^4$±7.5 x 10^3 for Mugilcephalas and $2.5 x 10^3$ to $2.3 x 10^5$

with an average of 7.5 x 10^4 ±2.8 x 10^3 /g for frozen Mackerel. In general, *Ps. multophilla* and *Ps. stutzeri* failed to be isolated from the examined samples Mackerel and Horse mackerel and *Ps. acidovorans* failed to be detected in the examined samples of in Horse mackerel. In contrast, most examined samples of frozen fish were highly contaminated by *Ps. fluorescents*, *Ps. putida* and *Ps. alicaligens*. Nearly similar percentages were recorded by Abd El Rahman (1996), Amany (1997), Bly *et al.* (1997) and Iman (2004) who revealed that incidence of Ps. flouresens (36.9%), Ps. alcaligens (15.5%) and Ps.anguillisoptica (18.5%). Lower results were obtained by Farag (1991) who found the incidence of Ps. fluorescens was 26.31%. Pseudomonas species are widely distributed in nature, unsanitized equipment's, pouted water and fishermen hands especially during harvesting, transportation and storage are considered as the source of fish contamination. The contamination of Pseudomonas organisms may be attributed to the heavily contamination boats and boxes which transfer the organisms to fish during cleaning (Frazier and Westhoff, 1987 & Venugopal, 1990*).* Accordingly, the consumption of such frozen fish contaminated with different members of psychrotrophic bacteria particularly and Pseudomonas species may constitute, at times public health hazards.

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البكتريا المحبة للبرودة في الاسماك المجمدة مع التركيز على بكتريا السودوموناس

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الملخص العربي

نم جمع عدد 90 عينة من ثلاثة أنواع من الاسماك المجمدة بالأسواق المصرية هي المكرونة الماكريل والهورس ماكريل بواقع 30عينة من كل نوع وقد اوضحت النتائج ان متوسط العدد الكلى للميكروبات المحبة للبرودة $4.08 \times 10^{5} \pm 0.71 \times 10^{5}$ في أسماك المكرونة و$9.95 \times 10^{4} \pm 2.13 \times 10^{4}$ في أسماك الماكريل و$3.66 \times 10^{4} \pm 0.49 \times 10^{4}$ في أسماك الهورس ماكريل. ومتوسط العدد الكلى لميكروبات السودوموناس. $1.26 \times 10^{4} \pm 0.32 \times 10^{4}$ للمكرونة ، $5.18 \times 10^{3} \pm 0.91 \times 10^{3}$ للماكريل ، $2.47 \times 10^{3} \pm 0.55 \times 10^{3}$ للهروس ماركريل على التوالي . أيضاً تم عزل ميكروبات السودوموناس بنسب مختلفة وهي: 76.67 %، 66.67%، 63.33% من عينات المكرونة والماكريل والهورس ماكريل على التوالي. وقد دلت النتائج على أن الفروق ما بين عينات الأسماك المجمدة التي تم فحصها سواء كانت للميكروبات المحبة البرودة أو لميكروبات السودوموناس، كانت فروق جوهرية. وقد أكدت نتائج هذه الدراسة أن أسماك المكرونة أكثر تلوثاً بكلا من الميكروبات المحبة للبرودة وميكروبات السودوموناس ثم يليها أسماك الماكريل ثم الهورس ماكريل. هذا وقد تم مناقشة الأهمية الصحية للميكروبات المعزولة وتحديد مصادر تلوث الأسماك بها مع وضع بعض التوصيات لتحسين جودتها.

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