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QUALITY ASSURANCE OF IMPORTED FROZEN FISHES IN EGYPT

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SUMMARY

Twenty five imported frozen fish samples each of Horse mackerel, Mackerl, Mugel and Tilapia were tested for determination of Moisture content, Ph value, Rancidity and Total volatile basic nitrogen. Aerobic mesophilic bacterial count, Coliforms, Pseudomonas and Staphylococcus aureus counts, as well as isolation of Clostridium perfringens, E. coli, Pseudomonas, Salmonellae, and Vibrio-parahaemolyticus were investigated. The results in the order mentioned above were:

Moisture percentage: 68.96%, 68.97%, 72.62%, and 74.20%, Ph value: 6.13, 6.08, 6.14 and 6.21, Rancidity quantitatively:0.069, 0.069, 0.066 and 0.061, Total volatile basic nitrogen: 21.52, 21.04, 21.36 and 20.92.

Aerobic mesophilic bacterial count: $3x10^4$, $4.4x10^4$, $1.9x10^6$ and $2x10^6$, Coliform count: $20.4.1x10^2$, 50.4 and 24.7, Pseudomonad count: $1.1x10^3$, $1.8x10^3$ and $5.5x10^3$, Staphylococcus aureus count: $8.5x10^2$, $1.8x10^4$, $4.8x10^2$ and $1x10^3$, Clostridium perfringens: 8 isolates (32%), 6(24%), 3(12%) and 7(28%), E.coli: 9(36%), 12(48%), 6(24%) and 14 (56%). A total of 41 E. coli isolates, 12 (29.3%) were identified as E.coli biovar 1 and 29 (70.7%) biovar 11, Pseudomonas 13(52%), 13(52%), (15(60%) and 17(68%). A total of 58 Pseudomonas isolates, 9 isolates were Ps. aeruginosa (15.51%), 11 isolates each of Ps. alcaligens, Ps. flourescens, flavo (18.96%) of each and Ps. cepacia and Ps. putida (8 isolates/13.79% of each). Staph. aureus 2(8%), 3(12%), 0.0 and 3 (12%).

None of the examined samples yield neither salmonella nor vibrio-parahaemolyticus.

INTRODUCTION

Fish and fishery products could be the vehicle for all important types of food poisoning bacteria which include in addition to Salmonellae, Staphylococci and *Clostridium botulinum*, the so called non-specific group of microorganisms such as *E.coli*, Proteus species, *Clostridium perfringens* and *Bacillusus cereus*. (Venkataraman and Sreenivasan, 1953; Shewan, 1962; Lotfi et al., 1972; Artemchuk, 1976 and Souter et al., 1976). These bacterial infections are due to contamination of the product during handling, processing, storage, distribution or preparation for consumption, which is usually more frequent in countries where sanitary conditions are generally poor.

Freezing of fishes retards bacterial growth almost entirely. Some bacteria will survive however, because of the protective effects of fats and proteins may be involved in subsequent spoilage (FAO/WHO, 1974).

Although the aerobic plate count of any food article is not a sure indicative of its safety for consumption, yet it is of great importance in judging the hygienic conditions under which it has been produced, handled and stored (Levine, 1961).

Presence of *Staphylococcus aureus* in a processed food indicates its post processing contamination from the skin, mouth or nose of workers handling the food and inadequately cleaned equipments. (Thatcher and Clark, 1975).

Epidemiological studies have established vibrio-parahaemolyticus as a worldwide agent of gas-

troenteritis, and results of ecological studies demonstrate that it can be isolated from seafood (Josept et al., 1983).

The development of rancidity in fish has been attributed to the atmospheric oxidation of the fish oils. This process involves the formation and the decomposition of peroxides (Borgstrom, 1961).

Volatile base nitrogen has been used to determine early stages of spoilage of frozen fish, were the bacterial count and pH value were unsatisfactory (Paladino, 1943; Horie and Sekine, 1954; Wittforgel, 1958; Jendruch, 1967 and Kietzmann, 1967).

The initial post mortem pH value were considerably lower than 7.0 and extensive bacterial spoilage may occur with only small rise in pH value (Tarr, 1949).

Therefore, the present investigation was initiated to determine the chemical and bacteriological quality of four different kinds of imported frozen fishes.

MATERIAL AND METHODS

A total of one hundred random frozen fish samples, of Horse mackrel, Mackrel, Mugel and Tilapia (25 samples each) were submitted to Department of Food Hygiene-Animal Health Research Intitute (Dokki-Giza) in closed cartons, muscles were obtained separately in sterile plastic bags and subjected immediately to the required chemical and bacteriological examinations. Handling and preparation of the collected samples were carried out according to (Thatcher and Clark, 1975 and Bailey and Scott, 1978).

1. Chemical examination

a. Determination of pH value was carried out by method recommended by Dodge and Stadelman (1960).

b. Determination of moisture content was applied according to the method reported by Jaocobs (1951).

c. Determination of rancidity using kreis test (quantitatively) according to the method recommended by Amer et al. (1975).

d. Determination of total volatile basic nitrogen (TVBN) according to FAO (1980).

II. Bacteriological examiantion:

A. Bacterial count:

Deterimination of aerobic mesophilic bacterial count using plate count technique on nutrient agar, staphylococci count using surface spread plate method on Baird-parker agar, pseudomonas count using surface spread plate method on pseudomonas selective agar and coliforms count (MPN) using multiple tube fermentation technique in lauryl sulphate tryptose broth tubes, were carried out as recommended by ICMSF, 1980.

B. Isolation and identification of certain pathogens:

Isolates suspected to be clostridium, pseudomonas, salmonellae, staphylococi and vibrio-parahaemolyticus were identified biochemically according to the techniques recommended by Edwards and Ewing (1972); Cowan and Steel (1975); Wilsons and Miles (1975) and Merchant and Paker (1983), while E.coli isolates were biotyped according to Stiles and Laiking (1981) and Mehiman and Romero (1982).

RESULTS AND DISCUSSION

From the results achieved in (Table 1) the mean value of moisture content was higher in Tilapia (74.2%), followed by Mugil (72.67%), Mackrel (68.9%) and Horse mackrel (68.7%). Concerning the mean pH value, it was 6.13, 6.08, 6.13 and 6.12 in Horse mackrel, Mackrel, Mugel and Tilapia respectively. The mean value of rancidity was higher in Horse mackrel (0.069), followed by Mackrel (0.068), Mugel (0.066) and Tilapia (0.061). The mean value of total volatile nitrogen was 21.52, 21.36, 21.04 and 20.92 mg/100 gm.) in Horse mackrel, Mugel, Mackrel and Tilapia respectively.

The results of chemical examiantion recorded here are relatively more or less in accordance with the previously reported by several investigators

Table (1): Frequency distribution of Moisture content, pH value, Rancidity and Total volatile basic nitrogen (TVBN) of the examined frozen fishes samples.

Samples examined	Frequency distribution														
	Moisture %				pH value				Rancidity				TVBN		
Type	No.	Min.	Max.	Mean	S.E.	Min.	Max.	Mean	S.E.	Min.	Max.	Mean	S.E.	Min.	Max.	Mean	S.E.
Horse mackerel	25	62	74	68.96	±0.48	5.9	6.3	6.13	±2.31	0.052	0.098	0.069	±2.93	18	24	21.52	±0.29
Mackerel	25	66.2	72	68.97	±0.38	5.9	6.4	6.08	±2.52	0.052	0.094	0.069	±2.83	19	24	21.04	±0.62
Mugel	25	69	77	72.64	±0.46	5.9	6.4	6.14	±2.59	0.053	0.094	0.066	±2.54	19	25	21.36	±0.38
Tilapia	25	69	81	74.20	±0.47	5.9	6.5	6.12	±2.67	0.052	0.095	0.061	±1.78	19	24	20.92	±0.35

Table (2): Bacteriological quality of examined imported frozen fishes samples.

Sample examined	+ve samples	Aerobic mesophilic bacterial count			+ve samples	Coliform count			+ve samples	Pseudomonas count			+ve samples	Staphylococci count		
Type	No.	No.	%	Min.	Max.	Mean.	No.	%	Min	Max	Mean	No.	%	Min	Max	Mean
Horse mackrel	25	25	100	2.5×10^{4}	1.0×10^{5}	3.0×10^{4}	14	56	0	210	20.4	13	52	50	4×10^{4}	1.1×10^{5}	7	28	1.1×10^{3}	5.6×10^{3}	2.5×10^{3}
Mackrel	25	25	100	2×10^{4}	5.0×10^{4}	4.4×10^{4}	17	68	0	1150	102	13	52	10^{2}	5×10^{4}	2.4×10^{5}	5	20	10^{2}	8.7×10^{3}	1.8×10^{3}
Mugel	25	25	100	5×10^{3}	1.9×10^{5}	9.3×10^{4}	11	44	0	500	50.4	15	60	10	3×10^{3}	1.8×10^{5}	9	36	1.3×10^{3}	2.4×10^{3}	4.8×10^{2}
Tilapia	25	25	100	9.3×10^{4}	2.0×10^{5}	1.2×10^{5}	20	80	0	150	27.4	17	68	40	5×10^{4}	5.5×10^{5}	6	24	2.6×10^{3}	2.6×10^{3}	1.0×10^{3}

(Tarr, 1941 and 1949; Bailey, 1950; Nagib, 1963; Abdel-Aty, 1971; Elmossalami and Sedik, 1973; Baradach and Priser, 1978 and El-Sayed, 1991).

Concerning the optical density limits for acceptance, it was stated by Amer et al. (1975) that optical density >0.085 is considered unacepted (signs of fat rancidity). The optical density increased gradually during frozen storage due to the formation of epihydrin aldhyd which was derived from oxidation of fat (Patton et al. 1951).

It is evident from (Table 2 & Fig. 1) that the mean value of aerobic mesophilic bacterial count/gram was highest in Tilapia (1.2×10^5), followed by Mugel (9.3×10^4), Mackrel (4.4×10^4) and Horse mackrel (3×10^4). Regarding the most probable number of coliforms count, the mean value and incidence were 20.4 (56%), 10^2 (68%), 50.4 (44%) and 24.7(80%) of the examined Horse mackrel, Mackrel, Mugel and Tilapia respectively. The average number of pseudomonas count was (1.1×10^3) in Horse mackrel, (2.4×10^3) in Mackrel, (1.8×10^3) in Mugel and (5.5×10^3) in Tilapia. The average number of staphylococci count was highest in Mackrel (1.8×10^4) in 20% of the examined samples followed by Tilapia (1×10^3) in 24%, Then Horse mackrel (8.5×10^3) in 28% and Mugel (4.8×10^2) in 36% of the tested samples. The results of aerobic mesophilic bacterial count, coliforms, pseudomonas and staphylococci counts recorded here are relatively more or less in accordance with the data reported by other investigators (Abdel-Hafeiz, 1991; FAO/WHO 1974; Levine, 1961; Saddik et al., 1985 and Shewan, 1962). In this respect, Shewan (1970) reported that after handling of frozen fishes, Aerobic Plate Count (APC), coliforms (MPN)

Staphylococci counts per gram were increased considerably than the suggested standards (APC > 10^5/gram, coliforms <$2x10^2$/gram and Staph. aureus 10^2/gram).

From the results achieved in (Table 3 & Fig.2) it is obvious that E.coli was isoalted from 9 samples (36%), 12(48%), 6(24%) and 14(56%) of the examined Horse mackrel, Mackrel, Mugil and Tilapia respectively.

On the other hand, *Staphylococcus aureus* was isolated from 3 samples (12%), 2 (8%) and 3 (12%) of the examined Mackrel, Horse mackrel and Tilapia respectively, while examined Mugil samples were negative for *Staph aureus. Clostridium perfringens* was isolated from 8 samples (32%), 6(24%), 3(12%) and 7(28%) of Horse mackrel, Mackrel, Mugil and Tilapia respectively. On the other hand, salmonellae as well as Vibrioparahaemolyticus could not be detected in any of the examined frozen fish samples. These findings are nearly extent to those recorded by FAO/WHO (1974), Saddik et al. (1985) and Ahmed et al. (1986).

Table (3): Incidence of Clostridium perfringens, E.coli, Salmonella, Staph. aureus and Vibrioparahaemolyticus in the examined frozen fishes samples.

Samples examined		Incidence of isolation of bacteria from examined samples									
Type	No.	Cl.perf.		E.coli		Sal.		S.aureus		V.parahaemo.	
		No.	%	No.	%	No.	%	No.	%	No.	%
Horse mackrel	25	8	32	9	36	-	0.0	2	8	-	0.0
Mackrel	25	6	24	12	48	-	0.0	3	12	-	0.0
Mugel	25	3	12	6	24	-	0.0	-	0.0	-	0.0
Tilapia	25	7	28	14	56	-	0.0	3	12	-	0.0
Total	100	24		41	-			8		-	

It is clear from Table (3) that non of the examined samples yielded neither Salmonellae nor V. parahaemolyticus.

In this respect, Saddik et al. (1985) failed to iso-

Table (4): Incidence and frequency of Escherichia coli isolates from different frozen fishes samples.

Types of examined samples	No. of tested isolates	Biovars of E.coli: Biovar I No.	Biovar I %	Biovar II No.	Biovar II %
Horse mackrel	9	1	11.1	8	88.9
Mackrel	12	3	25.0	9	75.0
Mugil	6	2	33.3	4	66.7
Tilapia	14	6	42.9	8	57.1
Total	41	12	29.3	29	70.7

Table (5): Frequency distribution of pseudomonas species isolated from examined frozen fish samples

Isolated strains	Frequency distribution of examined samples: Horse mackrel No.	%	Mackrel No.	%	Mugil No.	%	Tilapia No.	%	Total No.	%
Ps.aeruginosa	4	30.8	1	7.7	3	20	1	5.9	9	15.51
Ps.alcaligens	2	15.4	3	23.1	1	6.7	5	29.4	11	18.96
Ps.flourescens	1	7.7	1	7.7	5	33.3	4	23.5	11	18.96
Ps.cepacia	1	7.7	2	15.4	2	13.3	3	17.6	8	13.79
Ps.flava	4	30.8	3	23.1	1	6.7	3	17.6	11	18.96
Ps.putida	1	7.7	3	23.1	3	20.0	1	5.9	8	13.79
Total	13		13		15		17		58	

late salmonella fromt he examined fish samples, while Ahmed et al. (1986) isoalted 7(2.07%) coagulase positive Staph. aureus strains and two strains of Clostridium perfringens from the examined samples of Tilapia.

It is evident from Table (4) that out of 41 *E.coli* isolates, 12(29.3%) were identified as typical *E.coli* (biovar 1), 29 (70.7%) as atypical *E.coli* (biovar 11).

It is clear that *E.coli* biovar 1 was particularly high in Tilipia and Mugil, while biovar 11 was high in Horse mackrel and Mackrel.

The incidence and frequency of *E.coli* biovars recorded in frozen fishes in the present work in agreement with that previously reported by Surkiewicz et al. (1967) and Hassan (1987).

It is evident from Table (5) that out of (58) pseudomonas isolates, 9(15.51%) were identified as Ps. aeruginosa, 11 (18.96%) each of Ps. alcaligens, Ps. flourescens and Ps. flava and 8 isolates (13.79%) each of Ps. cepacia and Ps. Putida. The high frequency of pseudomonas species were recovered from Tilipia followed by Mugil, Horse mackrel and Mackrel.

Average Aerobic mesophilic, Coliforms, Pseudomonas and Staphylococcus counts in imported frozen fishes.

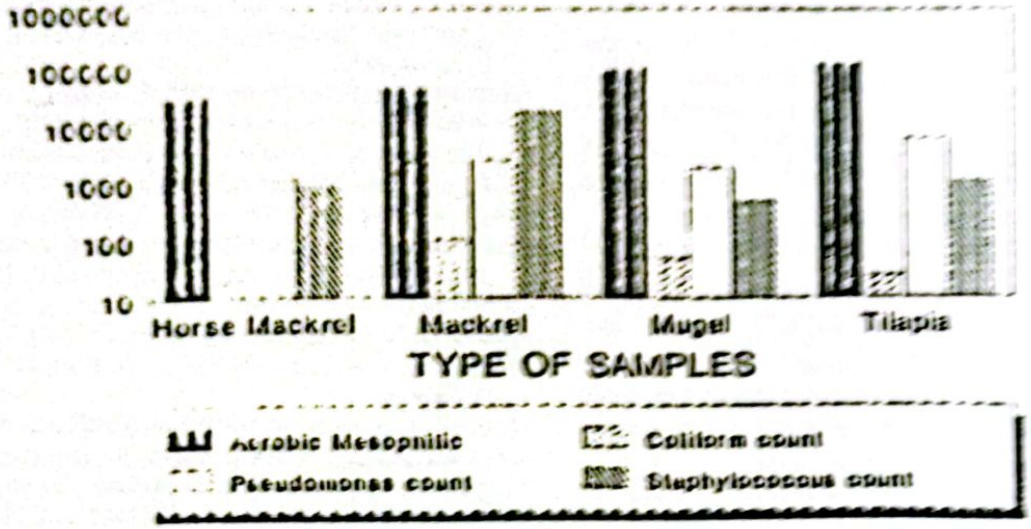

Fig.(1)

Incidence of Cl.perfringens, E.coli, Staph.aureus, Salmonellae and V.parahaemolyticus in frozen fish samples.

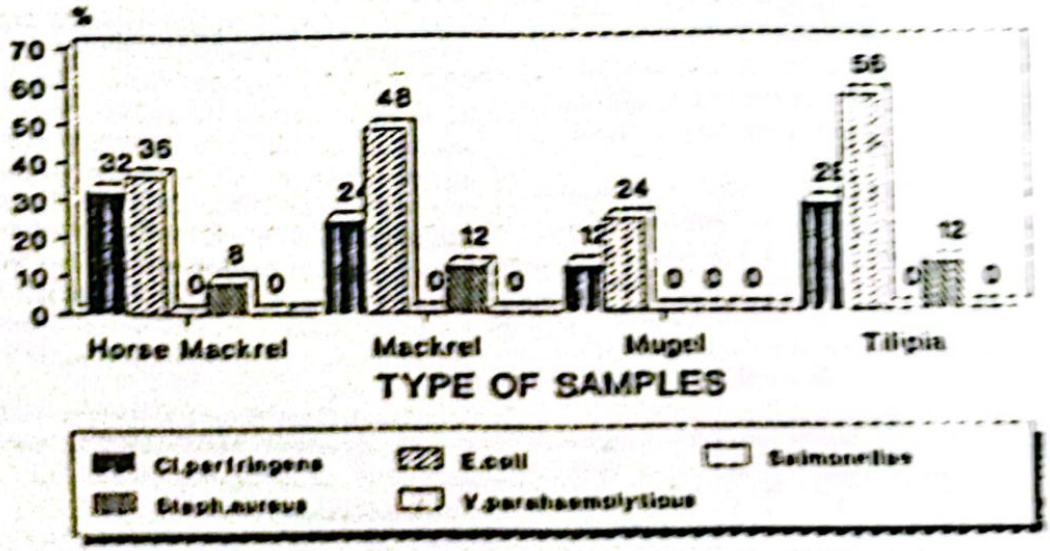

Fig.(2)

Damoglou and Downey (1984) isolated 12 strains of pseudomonas species capable of spoiling fish, one of these isoaltes (9.1%) was identified as Ps. flourescens. In this respect, **Ahne et al. (1982) and Gatti and Nigreili (1984)** isolated Ps. flourescens from the examined fish samples, they concluded that Ps. flourescens is a true fish pathogen. Furthermore, **Edward and Kraszewski (1991)** isolated 361 strains of Ps. species from fish, (30 isolates/8.3%) were identified as Ps. aeruginosa, (21/5.8%) Ps. flourescns, (18/4.9%) Ps. alcaligens and (1/0.3%) were identified as Ps. cepacia.

It is concluded from the present study, that 14 out of 100 imported frozen fish samples examined were unfit for human consumption due to either presence of rancidity and/or contaminated with Staphylococcus aureus, Clostridium perfringens and *E.coli*. Such results indicate improper hygienic measures adopted in place during fishing, handling, storage and shippment which may lead to public health hazard to the consumer specially if submitted to another load of contaminaition in door during transportation, storage and marketing.

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