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QUALITY MONITORING OF SOME FARM FISH MARKETED IN KAFR EL-SHEIKH GOVERNORATE

(With 4 Tables)

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تقييم جودة بعض الاسماك بمزارع كفر الشيخ

يحيي السيد على محمود

تناولت الدراسة فحص عدد ٣٠٠ عينة من الاسماك السليمة والمصابة ببعض الأمراض البكتيرية ولكنها من ناحية الكشف الظاهرى مقبولة للاستهلاك الآدمى بواقع ١٠٠ عينة من كل من أسماك البلطى والقرموط والبورى والمأخوذة من مزارع الأسماك المختلفة بمحافظة كفرالشيخ لتقييم حالتها البكتريولوجية والكيميائية. وقد دلت نتائج الفحص على أن متوسطات العدد لكلى للميكروبات الهوائية والميكروبات المحبة للبرودة والميكروبات المعوية فى الأسماك السليمة هى: ٣×١٠^٣ ± ١٢,٠×١٠^٣ ، ١,٦٨×١٠^٣ ± ١٢,٠×٨,٠×١٠^٢ ± ١,٧×١٠^٢ لكل جرام من عضلات الأسماك فى البلطى و٣,٠٣×١٠^٣ ± ٤٤,٠×١٠^٣، ١,٧٦×١٠^٣ ± ٠,٠٧×١٠^٣، ٣,٢٨×١٠^٢ ± ٣٣,٠×١٠^٣ لكل جرام من عضلات أسماك القرموط و١,٨٦×١٠^٣ ± ١,٠×١٠^٣، ١,٤٣×١٠^٢ ± ٦٥,٠×١٠^٢، ٢,٧٠×١٠ ± ١,٠×١٠ لكل جرام من عضلات أسماك البورى. بينما كان متوسط هذه المجموعات البكتيرية فى الأسماك المصابة بأمراض بكتيرية هي ٦,١٧×١٠^٥ ± ٣٢×١٠^٥، ٤,١٦×١٠^٤ ± ٤١,٠×١٠^٤، ٤,٨٠×١٠^٣ ± ٠,٢٢×١٠^٣ لكل جرام من عضلات أسماك البلطي و٦,٢٥×١٠^٥ ± ٢١,٠×١٠^٥ ، ٣,٧٥×١٠^٤ ± ٢١,٠×١٠^٤ ، ٦,٢٥ ×١٠^٣ ± ٢١,٠×١٠^٣ لكل جرام من عضلة أسماك القرموط و٢,٣٠×١٠^٥ ± ١٢,٠×١٠^٥ ، ١,٦٥×١٠^٤ ± ١٥,٠×١٠^٤ ، ٣,٦٠×١٠^٣ ± ١٩,٠×١٠^٣ لكل جرام من عضلة أسماك البورى على الترتيب. كما تم عزل ميكروبات الأيروموناس هيدروفيلا ، السودوموناس ايروجنبوزا ، ادواردزيلا تاردى ، فيبريوباراهيمولينكس ، الشرشياكولاى ، والسالمونيلا ،والليستريا ، والفلافوباكتريم والاكروموباكتر والكروموباكتريم والبرسينيا انيتروكوليتكا بنسب مختلفة. وكذلك بغض النظر عن حالة الصحة للأسماك فإن الفحص الكيميائى وضح أن متوسطات قيم الأس الأيونى الهيدروجينى كانت ٧,٠١±٠,٠١٨ ، ٧,٤±٠,٠١٣ وكذلك ٦,٩٨±٠,٠١٦ فى أسماك البلطى والقرموط والبورى على التوالى وكان متوسط المركبات النيتروجينية الطيارة ١٩,٦٠، ١٩,٧٣، ٢٣,١٣ مجم لكل ١٠٠ جرام

من عضلات أسماك البلطى والقرموط والبورى على التوالى. كما اهتمت الدراسة بتوضيح الأهمية الصحية للميكروبات المعزولة مع ذكر بعض التوصيات لضمان سلامة المستهلك.

SUMMARY

A total of 300 healthy and diseased fish samples representing *Tilapia nilotica, Clarias lazera* and *Mugil cephalus* (100 of each) were collected from different fish farms in Kafr El-Sheikh Governorate to evaluate their bacteriological and chemical qualities. The obtained results indicated that the mean values of Aerobic Plale Count (APC). Psychrotrophic and Enterobacteriaceae counts of healthy fish samples were $3 \times 10^3 \pm 0.12 \times 10^3$, $1.68 \times 10^3 \pm 0.12 \times 10^3$, $8.0 \times 10^2 \pm 1.76 \times 10^2$ bacteria per gram of muscle samples, for *Tilapia nilotica*. On the other hand, in case of *Clarias lazera* the mean values of the same bacterial groups were $3.03 \times 10^3 \pm 0.44 \times 10^3$, $1.76 \times 10^3 \pm 0.07 \times 10^3$ and $3.28 \times 10^2 \pm 0.33 \times 10^2$, per gram of muscles. While these mean values were $1.86 \times 10^3 \pm 0.10 \times 10^3$, $1.43 \times 10^2 \pm 0.65 \times 10^2$, and $2.70 \times 10 \pm 0.10 \times 10$ bacteria per gram of muscle samples of *Mugil cephalus*, respectively. In case of diseased fish muscle samples the results in *Tilapia nilotica* were $6.17 \times 10^5 \pm 0.32 \times 10^5$, $4.16 \times 10^4 \pm 0.14 \times 10^4$ and $4.80 \times 10^3 \pm 0.22 \times 10^3$, respectively. In *Clarias lazera* muscle sample the mean values were $6.25 \times 10^5 \pm 0.21 \times 10^5$, $3.75 \times 10^4 \pm 0.21 \times 10^4$ and $6.25 \times 10^3 \pm 0.21 \times 10^3$/gram muscle, respectively, while in case of muscle samples from diseased *Mugil cephalus* the mean values were $2.30 \times 10^5 \pm 0.12 \times 10^5$, $1.65 \times 10^4 \pm 0.15 \times 10^4$ and $3.60 \times 10^3 \pm 0.19 \times 10^3$ per gram muscle sample, respectively. *Aeromonas hydrophila, Pseudomonas aeruginosa, Vibrio parahaemolyticus, E. coli, Salmonella spp., Listeria spp. Flavobacterium, Achromobacter, Chromobacterium* and *Yersinia species* were isolated from examined samples with different percentages. Regardless the condition of the fish the overall mean values of pH and Total Volatile Nitrogen for muscle samples were 7.01 ± 0.018 & 19.6 ± 0.43 mg%, 7.04 ± 0.03 and 6.98 ± 0.016 & 19.78 ± 0.28 mg% and 6.98 ± 0.016 & 23.13 ± 0.12 mg% for *Tilapia nilotica*, *Clarias lazera* and *Mugil cephalus*, respectively. Furthermore, the public health significance of isolated bacteria and some recommendations to ensure consumer safety were discussed.

Key words: *Monitoring, Farm Fish*

INTRODUCTION

Many fish diseases especially those caused by bacteria as vibriosis, haemorrhagic septiceamia, gill disease of bacterial cause and diseases associated with skin lesions as discolouration, ulcers, haemorrhagic patches and frunclosis are considered of great importance for both of public health and the shelf of the fish (Inglis et al., 1993).

Okpokwasili and Obah (1991) stated that these diseases are considered of great public health significance as they could be cause disease condition for the consumer in the form of infection, bacterial food poisoning associated gastroenteritis. The disease acts as stress factor resulting in lowering of body resistance leading to invation of the fleshy parts with great amounts of different kinds of saprophytic bacteria localized normally in gastrointestinal tract gills and the surface slime coat of the skin (Hayes, 1986). Another important point stated by Shewan, 1971 was the bad setting and higher pH which is suitable for the growth and multiplication of bacteria with consequent bad keeping quality especially in long distances of transportation. In addition saling of such fish especially when the lesions of the disease condition are present will result in unacceptability from the consumer such fish are considered absolutely dangerous on the health condition of the consumer although if it was accepted by organoleptic examination and of economic loses especially in farms of intensive production Wong et al. (1967). The present result study was planned out to investigate the quality monitoring of some marketed fish in Kafr El Sheikh Governorate.

MATERIAL and METHODS

Samples collection:

Three hundred fish samples were collected from different fish farms in Kafr El-Sheikh Governorate. Samples were represented by three species of fish of *Tilapia nilotica*, *Clarias lazera* and *Mugil cephalus* (100 of each). Each fish species included healthy and diseased fish (50 samples of each). The diseased groups were appear by symptoms which are not attract the attention of consumer (i.e. apparently healthy). The collected samples were transferred to the laboratory under complete hygienic condition with as possible minimum time of delay. They were subjected to the following examination.

A) **Organoleptic examination**: were carried out according to Braumuller (1958). The results were recorded and calculated in Table (1).

B) Bacteriological examination:

a. Preparation of Samples and TAPC (ICMSF, 1978):

5 grams of fish was taken from the dorsal muscles after immersion the fish in alcohol 70% and flaming its surface as well as rusting of the area of the skin at the site of sampling. Under complete sterile condition the intermediate dorsal muscle layer were taken. Using stomacher the sample was mixed well with 45 ml of sterile Peptone water 1%, then, a decimal dilution using sterile Peptone water 1%, sterile test tubes and sterile pippets. 1 ml from each dilution were inoculated into sterile duplicated sets of petri-dishes. Then, Standard Plate Count Agar melted and tempered at 45° C ± 1° C was poured on each inoculated dish about 15 ml. Well mixing by tilting and circuling movement. After the deheshes solidified they were incubated at 35 ± 1° C for 24 hrs (ICMSF, 1978) after incubation the results recorded, calculated and statistically tabulated.

b. Psychrotrophic count: (ICMSF, 1978) using pouring technique and Standard Plate Count Agar similar procedures as in total aerobic plate count but the incubation at 7° C for 10 days (ICMSF, 1978). Results were recorded and calculated.

c. Total Enterobacteriaceae Count: (Gork, 1976) using Violet Red Bile Glucose agar.

d. Isolated and Identification of bacteria: Carried out according to modified Vanderzant and Nickleson (1969) identification sckems. The frequency distribution for each isolated bacteria were recorded and tabulated depending on the number of the samples.

B) Chemical examination:

Determination of pH:

Determination of pH (Hydrogen Ion Concentration) of fish muscle sample according to Pearson (1984) using Digital pH meter C.D. 620).

Determinaiton of TVN:

Determination of Total Volatile Nitrogen in fish muscles of examined samples using Conway's Microdiffusion Method (1957).

RESULTS

The results are obtained at Tables (1, 2, 3 and 4)

DISCUSSION

A) Organoleptic examination:

The present results in Table (1) revealed that the organoleptic examination of the collected healthy fish samples were 64.2%, 71.4% and 78.5% of *Tilapia nilotica*, *Clarias lazera* and *Mugil cephalus*, respectively; they were considered fit for human consumption according to the Braumuller Scale (1958). While in case of the diseased fish samples (apparently healthy collected fish samples were 34.2%, 42.8% and 50.0%, respectively, in case of *Tilapia nilotica*, *Clarias lazera* and *Mugil cephalus*. They were considered of border line i.e. (rapid consumed samples) according to Braumuller Scale (1958).

B) Bacteriological examination:

The obtained results in Table (2) revealed that the Total Aerobic Plate, Psychrotrophic and Enterobacteriaceae counts in an overall mean were $3.1x10^5 \pm 0.35x10^5$, $2.16x10^4 \pm 0.28x10^4$ and $2.8x10^3 \pm 0.24x10^3$, respectively in case of *Tilapia nilotica*, $3.14x10^5 \pm 0.33x10^5$, $1.96x10^4 \pm 0.21x10^4$ and $3.29x10^3 \pm 0.13x10^3$, respectively in case of *Clarias lazera* and $1.16x10^5 \pm 0.13x10^5$, $8.32x10^3 \pm 1.11x10^3$ and $1.81x10^3 \pm 0.2x10^3$, respectively, in case of *Mugil cephalus* (Table 2).

Regardless the type of fish, it was found that Aerobic Plate, Psychrotrophic and Enterobacteriaceae counts in case of healthy fish samples were $2.63x10^3 \pm 0.04x10^3$, $1.19x10^3 \pm 0.08x10^3$ and $3.81x10^2 \pm 0.65x10^2$, respectively. While in case of diseased fish samples were $4.91x10^5 \pm 0.20x10^5$, $3.19x10^4 \pm 0.18x10^4$ and $4.88x10^3 \pm 0.15x10^3$, respectively.

Moreover, it was found that significant variance ($P<0.05$) was recorded in case of all bacterial counts of healthy and diseased fish. Also a significant variance appeared between the different types of fish samples.

There was a significant difference between all bacterial counts within the same conditions and within the same type.

Results obtained were nearly similar to those reported by Hunter (1920) who recorded that the bacterial counts from the belly walls of disordered salmon was about two or three times as for healthy ones. Shewan (1971) recorded that the counts of bacteria ranged between zero to 10^3 depending on the condition of environment in which the fish life, method of catching, degree of exhusion, stress of fish during catching, the type of fish, the type of muscle and the healthy condition of fish. Moreover, Jay (1986), Hayes (1992), Youssef et al. (1992), and Macphee et al. (1995) they stated that, as is the case for meat animals the inner tissues of healthy fish are sterile. The present result in Table (3) revealed that the frequency distribution of isolation and identified bacteria from muscles of three types of fish samples under healthy and diseased condition with different percentages they were, *Acromonas hydrophila, Pseudomonas aerogenosa, Yersinia enteroclotica* and *Yersinia rukri, Edwardsiella tarda, Vibrioparahaemolyticus, Escherichia coli, Salmonella spp. Flavobacterium, Chromobacterium* and *Achromobacterium* and *Listeria spp.*

Most of these bacteria are considered as a disease problem in fish, and little of them considered spoilage agent, although if they are present in high number can lead to health problems for the consumer from the public health point of view.

Inglis et al. (1993) reported that it is important to recognized the potential health hazard with which fish might be associated, so that they can be reduced as far as possible. Some bacterial infections of fish and fish products may directly influence human health and classified human infection caused by bacteria in fish into food poisoning and gastroenteritis due to *Salmonella spp. Vibrio Spp.* and *Clostridium spp.*

C) Biochemical examination:

The results in table (4) revealed that the pH values of healthy fish samples in an overall mean were 6.98 $\pm$ 0.009 and 7.03 $\pm$ 0.02 in diseased ones, regardless the type of fish. While the Total Volatile Nitrogen in relation to the condition of fish samples was 19.24 $\pm$ 0.3 mg percent in case of healthy fish samples and 22.4 $\pm$ 0.009 mg percent in diseased fish samples.

Concerning the type of fish, the Total Volatile Nitrogen in an overall mean were 19.6 $\pm$ 0.43, 19.73 $\pm$ 0.28 and 23.13 $\pm$ 0.17 mg percent in case of *Tilapia nilotica, Clarias lazera* and *Mugil cephalus*, respectively, (table 3).

Their was a moderate significant differences between all treatments in case of the pH values, while it was highly significant in case of Total Volatile Nitrogen.

The results agreed with Ayres et al. (1980), Jay (1986) and (1996), Hayes (1992) and Inglis et al. (1993).

Reay and Shewan (1949) stated that the pH of muscles of fish depends on the state of fatigue of the animal immediately perior to killing or more specifically to the muscle glycogen available for degradation to lactic acid Ellist (1947) reported that, catching method, season, area of capture, bacterial load, types of bacteria, healthy condition, degree of exhaustion and handling after catching. Moreover, Fraizier and Westhoff (1988) stated that the pH of the flesh of fish has an important influence on its perishability, not only because of its influence on the growth of bacteria. Wong et al. (1967) reported that Total Volatile Nitrogen present in the fish are commonly used as indices of fish spoilage.

Lang (1979) concluded that fish is considered fresh if the TVN is less than 20 mg percent and if it 30 mg percent considers state, whilst.

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Table (1): Organoleptic examination of examined fish.

Condition of fish	Ideal	Total	Healthy samples			Diseased sample		
Type of fish	degree	ideal degree	No. x degree		Percent	No. x degree		Percent
Tilapia nilotica	50 x 28	1400	50 x 19	950	64.2%	50 x 10	500	34.2%
Clarias lazera	50 x 28	1400	50 x 20	1000	71.4%	50 x 12	600	42.8%
Mugil cephalus	50 x28	1400	50 x 22	1100	78.5%	50 x14	700	50%

50 degrees or more — Fit for consumption
25-50 degrees — Border line or (rapid consumption).
25 or less degrees — Decomposed and unfit for consumption.

Table (2): Mean values of bacterial counts in examined fish.

Condition of fish	Healthy	Disease	Overall mean
Type of fish	Mean ± S.E.	Mean ± S.E.	± S.E.
• *Tilapia nilotica*			
T.A.P.C.	$3.0 \times 10^3 \pm 0.12 \times 10^3$	$6.17 \times 10^5 \pm 0.32 \times 10^4$	$3.1 \times 10^5 \pm 0.35 \times 10^5$
T.Ps.C.	$1.68 \times 10^3 \pm 0.12 \times 10^3$	$4.16 \times 10^4 \pm 0.41 \times 10^4$	$2.16 \times 10^4 \pm 0.28 \times 10^4$
T.E.C.	$8.0 \times 10^2 \pm 1.76 \times 10^2$	$4.80 \times 10^3 \pm 0.22 \times 10^3$	$2.80 \times 0^3 \pm 0.24 \times 10_3$
• *Clarias lazera*			
T.A.P.C.	$3.03 \times 10^3 \pm 0.44 \times 10^3$	$6.25 \times 10^5 \pm 0.21 \times 10^5$	$3.14 \times 10^5 \pm 0.33 \times 10^5$
T.Ps.C.	$1.76 \times 10^3 \pm 0.07 \times 10^3$	$3.75 \times 10^4 \pm 0.21 \times 10^4$	$1.96 \times 10^4 \pm 0.2 \times 10^4$
T.E.C.	$3.28 \times 10^2 \pm 0.33 \times 10^2$	$6.25 \times 10^3 \pm 0.21 \times 10^3$	$3.29 \times 10^3 \pm 0.136 \times 10^3$
• *Mugil cephalus*			
T.A.P.C.	$1.86 \times 10^3 \pm 0.1 \times 10^3$	$2.30 \times 10^5 \pm 0.12 \times 10^5$	$1.16 \times 10^5 \pm 00.13 \times 10^5$
T.Ps.C.	$1.43 \times 10^2 \pm 0.65 \times 10^2$	$1.65 \times 10^4 \pm 0.15 \times 10^4$	$8.32 \times 10^3 \pm 1.11 \times 10^3$
T.E.C.	$2.70 \times 10 \pm 0.1 \times 10$	$3.60 \times 10^3 \pm 0.19 \times 10^3$	$1.81 \times 10^3 \pm 0.2 \times 10^3$
• Overall mean ± S.E.			
T.A.P.C.	$2.63 \times 10^3 \pm 0.04 \times 10^3$	$4.91 \times 10^5 \pm 0.20 \times 10^5$	$2.47 \times 10^5 \pm 0.17 \times 10^{5(+)}$
T.Ps.C.	$1.19 \times 10^3 \pm 0.08 \times 10^3$	$3.19 \times 10^4 \pm 0.18 \times 10^4$	$1.65 \times 10^4 \pm 0.13 \times 10^{4(++)}$
T.E.C.	$3.81 \times 10^2 \pm 0.65 \times 10^2$	$4.88 \times 10^3 \pm 0.15 \times 10^3$	$2.63 \times 10^3 \pm 0.13 \times 10^{3(+++)}$

T.A.P.C. = Total Aerobic Count
T.Ps.C. = Total Psychrotrophic Count.
T.E.C. = Total Enterobacteriaceae Count
S.E. = Standard Error
\+ = Significant
++ = Moderately significant
+++ = Highly significant

Table (3): Frequency distribution of the isolated and identified bacteria of public health significant from the examined fish muscles.

Isolated bacteria	*Tilapia nilotica*		*Clarias lazera*		Mugil cephalus		Total	
	No.	%	No.	%	No.	%	No.	%
Aeromonas hydrophila	6	3%	6	3.0%	7	3.5%	19	12.67%
Pseudomonas aerogenosa	7	3.5%	5	2.5%	6	3.0%	18	12.00%
Yersinia ruckri	3	1.5%	6	3.0%	7	3.5%	16	10.66%
Edward siella	4	2.0%	5	2.5%	6	3.0%	15	10.00%
Vibrio parahaemolyticus	6	3.0%	7	3.5%	8	4.0%	21	14.00%
Salmonella species	2	1.0%	3	1.5%	1	0.5%	6	4.00%
Escherichia coli	5	2.5%	4	2.0%	3	1.5%	12	8.00%
Listeria spp.	1	0.5%	1	0.5%	1	0.5%	3	2.00%
Flavobacterium	5	2.5%	3	1.5%	2	1.0%	10	6.67%
Achromobacter	5	2.5%	4	1.0%	3	1.5%	12	8.00%
Chromobacterium	5	2.5%	4	2.0%	4	2.0%	13	8.67%
Yersinia enterocolotica	1	0.5%	2	1.0%	2	1.0%	5	3.33%
Total	50	100%	50	100%	50	100%	150	100%

No. of isolates and percent calculated depending of the number of fish collected samples.

Table (4): Mean pH and TVN of examined fish.

Condition of fish	Healthy	Disease	Overall mean
Type of fish	Mean ± S.E.	Mean ± S.E.	± S.E.
• *Tilapia nilotica*			
pH	6.99 ± 0.02	7.01 ± 0.03	7.01 ± 0.018
TVN	15.79 ± 03.4	23.4 ± 0.21	19.6 ± 0.43
• *Clarias lazera*			
pH	7.01 ± 0.01	7.07 ± 0.03	7.04 ±0.013
TVN	19.35 ± 0.45	20.7 ± 0.21	19.73 ± 0.28
• *Mugil cephalus*			
pH	7.01 ± 0.01	7.07 ± 0.03	6.98 ± 0.016
TVN	22.27 ± 0.24	23.70 ± 0.21	23.13 ± 0.17
• Overall mean ± S.E.			
pH	6.98 ± 0.01	7.03 ± 0.02	$7.00 \pm 0.01^{(++)}$
TVN	19.24 ± 0.30	22.40 ± 0.01	$20.82 \pm 0.20^{(+++)}$

++ = Moderately significant
+++ = Highly significant
pH = Hydrogen Ion Concentration Values
TVN = Total Volatile Nitrogen mg/100 gm of muscle