



Assessment of the Microbial Quality of Five Types of Iraqi Fresh Fish in Sulaimania markets.

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Abstract:

The aim of this study to assess the quality of five types of local, most heavily traded and widespread in Sulaimani, fresh fish including Grass carp (*Ctenopharyngodon idella*), Silver carp (*Hypophthalmichthys molitrix*), Common carp (*Cyprinus carpio*), Bizz (*Barbus esocinus*) and Shabbout (*Barbus grypus*), for the period from 1st February 2010 to 1st October 2010. Analyses were achieved on a sample of twelve fishes for each type; their weight ranged 2-3Kg. different standard inspection tests were applied. Microbiology evaluation: total plate count (TPC), Psychrophilic bacterial count and Psychrotrophic counts were within the acceptable limits for all types (TPC valued 8.37 For Bizz to 25.80×10^5 Cfug for Silver carp, Psychrophils valued 6.83 for Bizz to 63.91 $\times 10^5$ Cfug for silver carp and Psychrotrophs valued 7.7 for Bizz to 20.70×10^5 Cfug for Silver carp) but they varied in count. Wild inhabitant showed significantly better microbiology evaluation results than farmed among farmable types. Bizz and Shabbout have been sold from licensed markets had better significantly than those unlicensed markets. *Pseudomonas* spp. was detected in all five types in a range of 45.75 for Shabbout to 59.16×10^2 Cfug for silver carp with insignificant differences. Randomly selected *Pseudomonas* colonies revealed isolation of *P.aeruginosa* and *P.putida*.

Key word: local fresh fish; microbiology; quality; Sulaimani.

I. Introduction:

One of the most highly perishable food products is fish; during handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product [1]. The quality of fish could be degraded through a complex process, in which the physical, chemical and microbiological forms of deterioration are involved; enzymatic and chemical reactions

are usually responsible for the initial loss of freshness whereas microbial activity is responsible for the obvious spoilage and thereby establishes product shelf life [2]. Microorganisms exist on the skin/slime, gills and the gut of live and newly caught fish. According to Huss [3] the proportion of microorganisms commensal the fish on the surface and gills/ guts of fish is 10^2 - 10^7 colony forming units (cfu) /cm² and between 10^3 and 10^9 cfu/g respectively.

Spoilage of fish under aerobic conditions becomes apparent when specific spoilage bacteria (SSO) reaches the values of 10^8 - 10^9 /g flesh [2]. Normally, this occurrence is after a lag phase, the time-span of which mainly depends on the temperature before the bacteria enter into exponential growth particularly in tropical fish species stored under low temperatures (0°C) [4]. The major changes in fish freshness such as unattractive change in food characteristics such as, flavors and odors and color are largely due to bacterial growth and activity [5]. Microbiological methods are used to estimate bacterial numbers, in order to determine fish freshness, hygiene and or evaluate the possible presence of bacteria

II. Materials and methods:

This study was conducted in the laboratories of the Faculty of Agriculture science / University of Sulaimani/ Iraq, for the period from 1st February 2010 to 1st October 2010. Five types of fish most commercially traded and widespread in Sulaimani governorate, which were grass carp, silver carp, common carp, Bizz and Shabbout, were chosen randomly from two fish retail outlet, licensed (M1) and unlicensed (M2) markets. Analyses were achieved on a sample of twelve fishes for each type, their weight ranged 2-3Kg. Grass, Silver, and Common carps, were of two sources (farmed and wild), while Bizz and Shabbout were wild only. From each fish, five flesh specimens, 100 gram for each from different parts of the fish were obtained; scales were removed with skin by a sterile scalpel, flesh were picked out from five parts, cut into smaller pieces, mixed together and put in a sterile plastic bag, and stored in a deep freezer (-40°C) for chemical analysis.

or organisms of public health importance [6] Microbiological prediction/estimation of bacterial numbers therefore, in order to serve the purpose of food safety and shelf life determination, is expected to relate quantitatively to the characteristics of the food during storage [7]. Thus, this study was aimed to assess the quality characteristics of local fresh fish by means of standard methodologies including:

1. Detection the specific spoilage and pathogenic organisms.
2. Assessment of the quality characteristics according to valid international and local regulations.

A. Microbiological tests

Sample preparation

To 25gram flesh, 225ml of Butterfield's phosphate diluent were added and homogenized, this is the first 10^{-1} dilution, series of dilutions were prepared in 9ml Butterfield's phosphate diluent until 10^{-7} .

One ml was removed from each dilution and put in 3 petri-dishes, Plate Count Agar (Biolife, Italy) was cooled in a water bath to $45 \pm 1^\circ\text{C}$ and poured into the petri-dishes, mixed gently by swirling or tilting each plate. Incubated for periods and temperatures according to the bacteria detected.

Total plate count were enumerated in standard plate count agar after incubation at 37°C for 48 h as described by [8]. Psychrophilic counts were enumerated in standard plate count agar after incubation at a $5 \pm 1^\circ\text{C}$ for 10 days as described by [9]. Psychrotrophic counts were enumerated in standard plate count agar after incubation at a $15 \pm 1^\circ\text{C}$ for 4-5 days as described by [9].

B. Pseudomonas spp. [10]

Plates (ceterimide agar) were Incubated in a $20 \pm 1^\circ\text{C}$ for 48 h. colonies on the duplicate plates from dilutions gave 30-300 colonies per plate were counted using a colony counter. Colony forming units (cfu)/ml were obtained by multiplying the colony number with the dilution. Those Colonies on ceterimide agar have (typically producing a small, rough colony) further analyzed biochemically then fully identified by VITEK[®] 2 SYSTEM.

C. Statistical analysis

All data were subject to one-way analysis of variance (ANOVA) using SPSS 18.0 and XL Stat program for Windows. Differences Between the means were tested by Duncan's multiple range tests. The level of significance was chosen at $P < 0.05$ and the results are presented as mean [11].

III. Results and discussion:

Total plate count (TPC), for Psychrophilic count (PPC) and Psychrotrophic (PTC) are shown in table I. TPC revealed that Bizz have significant differences to Silver and Common carp ($P < 0.05$) while there were insignificantly differences to Grass carp and Shabbout. In turn, Common and Silver carps have no differences between them; also there were insignificant differences between Grass carp and Shabbout. Although some of them were with no significant differences. Also there were no differences appeared among Common carp, Silver carp, Grass carp and Shabbout. Bizz, Grass carp and Shabbout had low TPC (8.37, 15.75 and 15.49×10^5 cfu/gram respectively). Silver and Common carps had high TPC. (25.80

and 21.71×10^5 cfu /gram respectively) (Table I).

The results of TPC for all five types were within the standard range ($5 \times 10^5 - 10^7$ cfu /gram) for fresh fish according to [12] and even lower than results mentioned by other researches of different countries [13]. The maximal limit of TPC of the Iraqi standards for fresh fish is 10^7 [14] so, all types were safe in this respect.

Other local reports have recorded similar evaluations of total plate count; [15], have shown 56 and 65×10^5 cfu /gram in carp and Sabour respectively, (16) found 270, 220, 231, 289 and 264×10^4 TPC in Shabbout, Carsean, Gattan, Tawnni and Bizz respectively. The result for other fish also revealed similar results; [17] found a range of $2.2 \times 10^4 - 4.5 \times 10^5$ cfu/ g in *Cyprinus caprio* and *Cybiium tritor*. The standard ranging bacterial count of the current and local and non-local studies demonstrated an acceptable quality. The high total plate count, which may exceed the standard maximal limits, may be related to bad handling and mechanical damages during fishing as well as the time of exposing fish to air [18]. Again, consumers experienced on choosing a good quality fresh fish and can differentiate good from spoiled one which lead retailer to avoid exhibiting spoiled fish, as spoilage is almost a result of high bacterial count. Microorganisms are important reason for spoilage because they break down the food into a form they can utilize. Therefore, food quality decreases and spoilage starts at this stage and estimation of the quality of food products relies on the quantification of total numbers of microorganisms [19]. TPC. is an indicator of quality, effectiveness of handling procedures, storage conditions and long storage time at chilled temperature. The factors that influence the

microbial contamination and growth include fish species, size, method of catch, handling on-board, fishing vessel sanitation, processing, and storage condition [20; 21].

With respect to Psychrophilic bacterial count (table, I), all types contained Psychrophils of submaximal standard count (10^6 cfu /gram fish meat) as mentioned by [22], Bizz, Grass carp, Shabbout and Common carp showed low Psychrophilic count (6.83, 12.73, 14.2 and 15.57×10^5 cfu /gram respectively) while high count was seen in Silver carp (63.91×10^5 cfu/gram). Except Silver carp, the other four types have insignificant differences. Similar findings were reported in local and abroad for fish, [16] recorded that Psychrophilic count in Shabbout, Carsean, Gattan, Tawnni and Bizz were 218, 196, 288, 245 and 223×10^4 cfu /gram flesh respectively, Al-Sheriffi *et al.* [15] reported values of 8.8×10^4 and 27×10^5 cfu for Psychrophils/gram in carp and Shabbout respectively. Koussemon *et al.* [17], study on *Cyprinus caprio* and *Cybium Tritor*, found that Psychrophils ranged between 2.2×10^4 - 4.5×10^5 cfu/gram, and Scherer *et al.* [23] counted (3×10^5 cfu Psychrophils/gram) in Grass carp. Psychrophils on fresh fish are mostly from water source, in contrast TPC which increases due to contamination by the environment, theoretically Psychrophils become decreased after fishing and extraction of fish from water, but the high psychrophilic count may indicate a pollutant water source.

As shown in table I, all five types of fish under study were lower than the maximal standard limits of Psychrotrophic bacterial count which is 10^9 cfu/gram meats [24]. The mean values of Psychrotrophic count for Bizz, Grass carp and Shabbout were low (7.7, 9.1 and 10.18

$\times 10^5$ cfu/gram respectively) while they were higher in Silver and Common carps (20.30 and 15.05×10^5 cfu/gram respectively). No significant differences among all species were found. Bojanic *et al.* [25] have shown that the Psychrotrophic bacterial count may be related to the level of bacterial contamination of fish which depended on the environment and bacterial quality of water where fish was caught. Psychrotrophs in water, from which fish were caught, and environment source which include mesophilis that can tolerate and grow in low temperatures. Psychrophils grow in isolation at the temperature of psychrotrophs (20°C). So that psychrotrophs, which are an expression for low temperature tolerating mesophiles and some of psychrophils, are within the acceptable levels of quality. On this basis psychrotrophes are of important value in assessing food quality with respect to microbial contamination which is almost of environmental source. The count of Psychrotrophic bacteria increased after prolonged storage time of the fish on ice [24] and so they are a good indicator for storage time. As shown in table I, *Pseudomonas*, which is responsible for the development of meat spoilage [26], counted, as mean value, 49, 59.16, 55.16, 55.33 and 45.75×10^2 cfu/gram meat for Grass carp, Silver carp, Common carp, Bizz and Shabbout respectively. There were no standard limits for *Pseudomonas* either locally or at the international level compares with. However, no significant differences in pseudomonas bacteria count were shown among the five types. In a study it was shown the count of *Pseudomonas* was $3.9 \log_{10}$ CFu/gram in fish meat [25]. *Pseudomonas* increase with the increase of storage time; as Papsdopoulos *et al.* [27] observed within

15 days of storage, the number of *Pseudomonas* reached to 10^7 cfu/gram fish meat. Colonies picked out randomly from the selective plates for identification of *Pseudomonas* (Citermide agar) to species level, it appeared that all colonies picked from plates of Grass carp, Silver carp, Bizz and Shabbout were *Pseudomonas aeruginosa* while 8 colonies picked out from Common carp were *P. putida* and one was *P. aeruginosa* (table, I). *Pseudomonas aeruginosa* is found in many natural and domestic environments including plants, soil and surface water, especially warm moist environments containing organic material or contaminated by human or animal waste [28]. *Pseudomonas aeruginosa* were found to cause disease for fish; [29], isolated *Pseudomonas* spp from internal organs of sea fish, while [28] mentioned previously that *P. aeruginosa* does not

survive in marine environment because of high salinity. However, isolation of *P. aeruginosa* from fish means that water polluted by the neighbor terrestrial environment. In samples collected from the local markets, the highest count of *Pseudomonas* spp. was found in Sarpunti fish (4.80×10^7 cfu/g) while the lowest count was in Kachki fish (1.31×10^4 cfu/g) Among the samples of super shop, the highest count was found in Tilapia (8.68×10^5 cfu/g) and lowest counts of *Pseudomonas* found in both Rui and Ayr fish (1.35×10^3 cfu/g) [30]. *Pseudomonas* spp. is frequently associated with fish and have been isolated from skin, gills and intestine. Their load is explained by the population density in water. In an aquaculture, especially *P. aeruginosa* and *P. fluorescens* have been considered opportunistic pathogenic species [31].

Table.I: Microbiological Assessment Of Five Types Of Local Fresh Fish.

Type of fish	Total plate count $\times 10^5$	Psychrophilic count $\times 10^5$	Psychrotrophic count $\times 10^5$	<i>Pseudomonas</i> count		
				Total count $\times 10^2$	Identification of some isolates selected	
					<i>P. aeruginosa</i>	<i>P. putida</i>
Grass carp	15.75 ab	12.73 b	9.1 a	49 a	12	-
Silver carp	25.80 a	63.91 a	20.70 a	59.16 a	12	-
Common carp	21.71 a	15.57 b	15.05 a	55.16 a	1	8
Bizz	8.37 b	6.83 b	7.7 a	55.33 a	12	-
Shabbout	15.49 ab	14.2 b	10.18 a	45.75 a	12	-

Mean having different letters in the same column are significantly different. The differences are at $P < 0.05$. The means are the average of 12 replicates.

A. Microbiological evaluation in farmed and wild sources of fish

The microbiological evaluation of the three farmable types (Grass, Silver and Common carps) for farmed and their wild counterpart are shown in table II. Significant differences ($P < 0.05$) were found in total plate count (TPC), Psychrophilic, Psychrotrophic and *Pseudomonas* count between wild and farmed Grass carp. In wild Grass carp they were 10.75, 9.80, 5.35×10^5 and 29.66×10^2 cfu/g respectively while in their farmed counterparts they were 20.75, 15.66, 12.96×10^5 and 68.33×10^2 cfu/g respectively.

Wild Silver carp counted 15.51, 17×10^5 and 44.66×10^2 cfu/g for TPC, Psychrophilic and *Pseudomonas* count respectively which are significantly different to their farmed counterparts that counted 36.10, 110.83×10^5 and 73.66×10^2 cfu/g meat respectively. There were significant difference in Psychrotrophic bacteria count between farmed (40.5×10^5 cfu/g) and wild (0.9×10^5 cfu/g) Silver carp were found.

In Common carp there were significant differences ($p < 0.05$) in TPC, Psychrophilic and Psychrotrophic count

between wild Common carp (11.76, 5.93 and 0.9×10^5 cfu/g respectively) and the farmed type (31.66, 25.21 and 40.5×10^5 cfu/g respectively). Insignificant differences were seemed for *Pseudomonas* counts between farmed and wild Common carp.

Results with respect to TPC, Psychrophilic, Psychrotrophic and *Pseudomonas* counts for the three types of carps of both farmed and wild sources, revealed good quality as they were within the standard limits [12; 22; 24]. The wild source of three types showed a better quality than farmed type. Indeed, the TPC is a good indicator for fish quality [32]. The Psychrotrophs count were low in wild sources of three types which indicate better quality than their farmed counterparts because Psychrotrophs are responsible for deterioration of fresh fish during storage in cooling and freezing [18]. These results revealed that fish farms exposed to contamination more than natural running water which may be due to feeding processes or farming conditions.

Table II: Microbiological Assessment Of Wild And Farmed Sources Of Three Types Of Local Fresh Fish.

Type of fish		Test			
		Total plate count $\times 10^5$	Psychrophilic count $\times 10^5$	Psychrotrophic count $\times 10^5$	<i>Pseudomonas</i> count $\times 10^2$
Grass carp	farmed	20.75 a	15.66 a	12.96 a	68.33 a
	wild	10.75 b	9.8 b	5.35 b	29.66 b
Silver carp	farmed	36.10 a	110.83 a	40.5 a	73.66 a
	wild	15.51 b	17.00 b	0.9 b	44.66 b
Common carp	farmed	31.66 a	25.21 a	29.5 a	59.16 a
	wild	11.76	5.93	0.59	51.16

b b a a

Means having different letters in the same column are significantly different within same types. The differences are at P<0.05. The means are the average of 6 replicates.

B. Microbiological indicators in licensed and unlicensed markets

Table, III, showed the microbiological assessment of five fish type in M1 and M2, there were significant differences (P<0.05) in TPC, Psychrophilic and Psychrotrophic counts between two markets for Bizz and Shabbout. No significant differences were recorded for Grass, Silver and Common carps between M1 and M2. No significant differences were found for Grass carp, Silver carp, Bizz and Shabbout for *Pseudomonas* count between M1 and M2. TPC, Psychrophilic and Psychrotrophic counts (as mean values) for Bizz in M1 were 4.38, 1.10 and 5.60×10^5 cfu/gm respectively while in M2 the counts were 12.36, 12.56 and 9.83×10^5 cfu/gm respectively. For Shabbout in M1

the counts were 5.06, 6.98 and 7.5×10^5 cfu/gm respectively while in M2 they were 25.91, 21.56 and 12.81×10^5 cfu/gm respectively. There was a significant difference between *Pseudomonas* counts (P<0.05) in Common carp between both M1 (32.66×10^2 cfu/gm) and M2 (77.66×10^2 cfu/gm).

Bacterial counts of Bizz and Shabbout from M1 were freshest due to lower total bacterial count even if M2 was also has not exceeded the maximal acceptable limits 10^7 cfu/gm [12]. The result indicated that both Bizz and Shabbout from M1 showed better freshness, due to low bacterial count, than Bizz and Shabbout from M2 which also not exceed the maximum limits 10^7 cfu/gm [18].

Table.III: Microbiological Assessment Of Five Types Of Local Fresh Fish From Licensed (M1) And Unlicensed (M2) Markets.

Type of fish		Type of test			
		Total plate count $\times 10^5$	Psychrophilic count $\times 10^5$	Psychrotrophic count $\times 10^5$	<i>Pseudomonas</i> count $\times 10^2$
Grass carp	M1	14.95 a	11.05 a	7.65 a	36.66 a
	M2	16.55 a	14.41 a	10.66 a	61.33 a
Silver carp	M1	22.73 a	20.61 a	1.55 a	49.50 a
	M2	28.88 a	107.21 a	39.85 a	68.49 a
Common carp	M1	20.15 a	14.36 a	5.14 a	32.66 b
	M2	23.28 a	16.78 a	24.96 a	77.66 a
Bizz	M1	4.38 b	1.10 b	5.60 b	50.66 a
	M2	12.36 a	12.56 a	9.83 a	60.00 a
Shabbout	M1	5.06 a	6.98 b	7.5 b	41.16 a

M2	25.91	21.56	12.81	50.33
	a	a	a	a

Means having different letters in the same column are significantly different within same types. The differences are at $P < 0.05$. The means are the average of 6 replicates.

IV. Conclusion:

We can conclude that all five types of fish under study were lower than the maximal standard limits. The wild source of three types showed a better quality than farmed type. The result indicated that both Bizz and Shabbout from license market M1 showed better freshness.

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