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Sensory and chemical changes associated with microbial flora of *Oreochromis niloticus* stored in ice

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Abstract: One batch of *Oreochromis niloticus* samples attained to 25 kg was examined periodically each two days intervals to asses the sensory, physical, chemical and microbiological parameters during ice storage along of it's validity. Samples under study kept their normal organoleptic characteristics under ice storage (at 0°C) for 10 days. The mean values for each of pH, TVB-N and TMA-N ranged from (5.82 ± 0.01 to 6.50 ± 0.01), (9.38 ± 0.11 to 30.03 ± 0.14) and from (2.78 ± 0.01 to 10.02 ± 0.03) respectively. The mean total aerobic, psychrophilic bacteria, hydrogen sulfide producing bacteria, Pseudomonas, proteolytic, and lipolytic bacterial counts were ranged from (3.5×10^3 to 2.2×10^6), (1.1×10^3 to 1.2×10^6), (7.8×10^2 to 1.6×10^4), (9.2×10^2, to 5.5×10^4), (8.8×10^2 to 3.1×10^4) and from (1.5×10^2 to 3.3×10^3) CFU/g respectively. The bacterial isolates in the examined fish samples were *Pseudomonas putida biovar B*, *Pseudomonas fluorescence*, *Shewanella putrefaciens*, *Aeromonas sobria*, *Aeromonas caviae* and *Aeromonas allscharophilia* with a different incidence. In conclusion, fish chilled to the temperature of melting ice (0°C) as soon as possible after capture and maintained at this temperature until it reaches the consumers.

Keywords: *Oreochromis niloticus*, sensory, chemical, microbial, quality

Introduction

Oreochromis niloticus considered one of an excellent quality fish characterized by poor fat and rich vitamins with firmly textured rusticity and good sensorial properties of flesh making it more suitable and an appetizing fish to the consumers. *Oreochromis niloticus* have relatively fast growth, good utilization of the artificial diets, and high tolerance to the adverse environmental conditions results in diseases resistance and easy breeding (Boscolo *et al.,* 2001; Corpei, 2001; Maregoni, 2006).

Shelf-life of fresh fish from harvesting time until human consumption is very important criteria for allowing a processor or a retailer to plan the time length for the product and market control. The rate of quality loss and the shortness of the shelf-life referred to a fact that the fish is perishable product, have a wide variety of species and composition. Moreover; fishing technique, fishing area, handling practices, preservation, storage time and temperature and qualitative and quantitative composition of fish microflora could also affect on the quality and shelf life (Connell, 1990; Burt and Hardy, 1992; Gram and Huss, 1996; Olafsdottir, *et al.,* 1997).

Deterioration in fish quality have the signs of slime formation, off odor discoloration, changes in the texture and taste with gas production. These spoilage indicators referred to a variety of biochemical, microbial, enzymatic and physical mechanisms (Connell, 1990; Daczkowska-Kozon, 1993). The microbial activities with the resultant accumulation of microbial metabolites plays the crucial role in the spoilage process whereas the harvested fish go to dead, exhibited collapse of the immune system, allows the proliferation and colonization of bacteria on the skin surface consequently migrate into the fish flesh (Burt and Hardy, 1992; Clancy *et al.,* 1995). The bacterial flora belonging to genus *Pseudomonas* is the most important among chilled fish spoilers followed by *Vibrio* species belonging to *Aeromonas* and *Achromobacter* (Shetty and Setty, 1990). The psychrophilic bacteria, exhibited proteolytic activities (Singh *et al.,* 1993), also they have the ability to produce the fat splitting enzymes lipases, which give rise to quality changes in the chilled stored food (Gobbett *et al.,* 1996).

Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA-N) content are the most chemical parameters used for determination fish quality. The levels of these compounds increased with the onset of spoilage. This chemical compound is the primary cause for the fishy odors, which increased as spoilage proceeds and show good correlation with sensory analysis (Ruiz-Capillas and Horner, 1999; Özoğul and Özoğul, 2000). Chilling and freezing besides other of traditional methods hinder the microbiological spoilage (Gould, 1996). Marketing studies indicated that consumer's preferred the fresh fish than the frozen one. Therefore, the extension of fish shelf life and maintaining the quality is essentially for allowing the market to get good profitability (Connell, 1990). Icing reduces temperature to about 0°C, which lowers the growth rate of spoilage and pathogenic microorganisms (Huss, 1995).

Loss of quality with shortness of the shelf life

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of the fresh *Oreochromis niloticus* are considered hazardous to the consumers due to the proliferation and colonization of either pathogenic or spoiler bacteria with accumulation of unpleasant metabolites. So fish may incriminate in different cases of food poisoning and illness depending up on the types and species of the bacteria; such case of shewanellosis, traveler's diarrhea, self-limiting gastroenteritis and bacteraemia. The patients with liver disease and the immunocompromised individuals appear to be at higher risk. Health hazardous was commonly associated with fish spoilage results in unhealthy and unmarketable fish consequently economic loss (Connell, 1990; Palumbo *et al.,* 1992; Morais *et al.,* 1997; FDA, 2001; Krsnik *et al*., 2002; Otsuka *et al.,* 2007). Regarding safety and quality of fish for consumers**,** the present study aims to assess the quality of chilled storage *Oreochromis niloticus* through the assessment of the sensory, physical, chemical and microbiological parameters from the point of catches to spoilage.

Materials and Methods

Samples collection

One batch of fresh Bolti*, Oreochromis niloticus*, weighed 25 kg, purchased as soon as they were caught from farm-harvested area at Bahr El-Baker, Port-Said Governorate, Egypt. Individual fish approximately ranged from 250-300 g. Samples washed under clean running tap water then placed in a clean box with an appropriate quantity of crushed iced with a ratio of 1:1 (w/w) for ice and fish respectively. Ten fish samples were carried out immediately after fish delivery to the laboratory within 8 hrs from the caught and periodically each two days intervals to asses the sensory, physical, chemical and microbiological parameters along of it's validity.

Sensory evaluation

Sensory evaluation of samples were conducted with the natural day light and in well-ventilated room using the quality index scheme (QIM) proposed by Rodrigues (2008). Panelists asked in appropriate scheme forms with descriptive terms employed the main five quality parameters of the organoleptic characters of the fish namely: general appearance (skin, scales, stiffness and flesh texture), eyes (clearance of cornea, pupil and eye form), gills (smell and color), muscle color and abdomen (internal abdominal wall).

Rating was assigned on a condition of three point's hedonic scale, from 0 to 2, where 0 represent the highest quality score and 2 represent the lowest one with total score of 19. The quality index ranged from 0-19 for *Oreochromis niloticus (*Larsen *et al.,* 1992; Rodrigues, 2008). Shelf life of samples calculated according to FAO (1995). The theoretical demerit curve has a fixed point at (0, 0) and its maximum has to be fixed as the point where the fish has been rejected by sensory evaluation. This straight line could distinguish between the fish at the start of the plateau phase and fish near the end of the plateau phase.

Measurement of pH

pH meter was calibrated according to the manufacture's instrument (JENWAY 3510 pH Meter -ENGLAND) using certified buffer pH 7.00 and pH 4.00. A representative 10 g of the flesh were chopped into clean blender jar and covered with 100 ml distilled water at 25˚C. The mixture was blended for 30 second on high speed then transferred to a beaker. pH of the clear fluid measured according to ISO (1999).

Chemical evaluation

Chemical evaluation based on the quantitative determination of the total volatile nitrogen (TVB-N) and trimethylamine (TMA-N) in triplicate manner as described by AOAC, (1998). 200 ml of 75% (v/v) aqueous trichloroacetic acid was added to 100 g of minced flesh sample and homogenized in blender for a minute at high speed. The homogenate centrifuged at 3000 rpm for 10 min at 4˚C and filtered through Whitman No. 3 filter paper. 25 ml of mixture transferred into the distillation flask, and then 6ml of 10% NaOH was added. Beaker containing mixture of 10 ml boric acid, 0.04 ml methyl and bromocresol green indicator placed under the condenser for the titration of ammonia. The boric acid solution turned green after 4 min distillation and titrated with 0.0317 sulphuric acid using 0.05 ml graduated burette. The levels of (TVB-N) and (TMA-N) expressed as mg N/100 g fish.

Bacteriological evaluation

A representative 25 g of each sample were transferred aseptically to a stomacher bag (Seward medical, London, UK) containing 225 ml of sterile 0.1% (W/V) peptone saline (0.1% peptone + 0.85% NaCl) and homogenized for 60 second with stomacher (Lab. Circulator Stomacher 400, Seward Medical, London UK) to obtain the original homogenate fluid. Decimal serial dilution up to 10^6 where the procedures recommended by APHA, (2002) were applied for bacteriological evaluation of samples. Enumeration of total aerobic count performed on standard plate count

agar (Oxoid; CM 325) with incubation at 30ºC for 48 h; meanwhile at 7ºC for 10 days to enumerate the psychrotrophic on the same medium. *Pseudomonas* agar (Oxoid; CM 0559) supplemented with CFC (Oxoid; SR0103) used for total *Pseudomonas* count and incubated at 25˚C for 2 days; while the hydrogen sulfide producing organisms enumerated on peptone iron agar (Difco; 0089-17) and incubated at 25˚C for 3 days. Skimmed milk agar 10% (Oxoid, CML 31) and tributyrine agar (Oxoid, PM4) used for assessment both of total proteolytic and lipolytic with incubation at 30ºC for 72 h for them both. All isolated colonies were calculated and expressed as colony forming units (CFU/g) per gram of fish. Up to 5 of the obtained typical colonies were transferred to tryptic soy agar (Difco, 0369-17) for biochemical identification.

Statistical Methods

One-Way ANOVA test was performed on the treatment means for each parameter studied using Statistical Package for Social Scientists (SPSS) for windows 16.0 (SPSS Inc., Chicago, IL, and USA) to examine whether there were significant differences in the parameters analyzed with storage time in ice. Correlations between the sensory, chemical and microbiological parameters were also done (SPSS, 2007).

Results and Discussion

The quality index method (QIM) based upon objective evaluation of certain attributes of raw fish using a point scoring system. The score for all attributes are then added to give an overall sensory score the so-called quality index which increases linearly with keeping time on ice. The total demerit score can be used to predict the remaining shelf time. The score given by the panelists according to QIM scheme for each parameter presented in Tables 1 and 2 and sensory scheme. The demerit points were calculated for each interval of two days storage where the index increase with the storage time. The panelists totally scored 2.33 ± 0.21 on the 2nd day of storage. A significant increase ($P<0.05$) in the scores was noticed as the storage on ice progressed. On 16th day, the total demerit point score was 18.67 ± 0.21 the skin was dull with loss of the faint colors stripes, loss of scales and softness of fish, the eyes completely opaque, milky, cloudy and sunken. Gills smell was rancid and sour while it's color appear brown. The muscle was dark rose and the protenium appear white yellowish with black spots. So this point considered the point of rejection of *Oreochromis niloticus* fish; time (16 days) and score (18.67 ± 0.21).

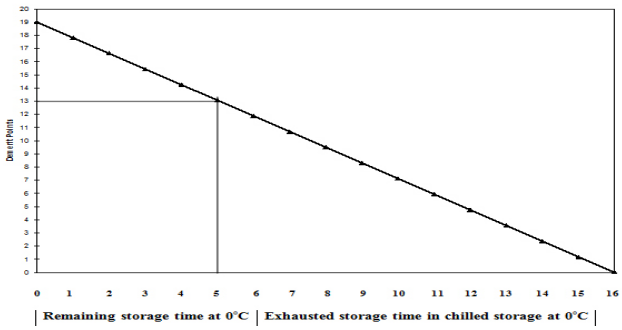

Figure 1. Predict the shelf-life and storage time remaining for *Oreochromis niloticus* samples under ice storage

The undesirable organoleptic changes were correspond to the values of each of pH, TVB-N and TMA-N, where the rates attained to 6.50 ± 0.01, 30.03 ± 0.14, and 10.02 ± 0.03 respectively. The changes in the levels of pH, TVB-N and TMA-N of the samples showed in Table 3; on the 0 day it's values were 5.82 ± 0.01, 9.38 ± 0.11 and 2.78 ± 0.01 respectively. During the chilled storage, there no significant changes in the pH, TVB-N and TMA-N up to the 8th day storage. Meanwhile on 16th day (rejection time) the levels attained to 6.50 ± 0.01, 30.03 ± 0.14 and 10.02 ± 0.03 respectively. These chemical parameters increased significantly along the 2nd week. The increase in pH levels with regard to increase in volatile bases and accumulation of ammonia due to decomposition of nitrogenous compounds by the microbial activities. These results agree with that previously reported by Pacheco-Aguilar, *et al.,* (2000); Erkan and Ozden (2008). The different recorded bacterial counts in Table 4 which increased scientifically during the storage time correlate with those organoleptic changes. These results showed that the deterioration in the quality of *Oreochromis niloticus* fish as evidence by the considerable values of these factors were in agreement with results recorded by Ruiz-Capillas and Moral (2001); EL-Mossalami *et al.* (2004) and Oyelese (2006). In addition, pH inversely influence on bacterial growth.

The initial bacterial counts of aerobic bacteria, psychrophilic bacteria and hydrogen sulfide producing bacteria, *pseudomonas*, proteolytic and lipolytic bacteria on 0 days (0-8 hr of catches) were 3.5 x 10^3 ± 1.8 x 10^2, 1.1 x 10^3 ± 3.7 x 10^1, 7.8 x 10^2 ± 2.7 x 10^1, 9.2 x 10^2 ± 2.7 x 10^1, 8.8 x 10^2 ± 2.8 x 10^1, and 1.5 x 10^2 ± 1.8 x 10^1 CFU/g of fish muscle respectively. While that on 16th day (rejection time) storage were 2.2 x 10^6 ± 5.6 x 10^4, 1.2 x 10^6 ± 9.2 x 10^4, 1.6 x 10^4 ± 3.7 x 10^2, 5.5 x 10^4 ± 1.8 x 10^3, 3.1 x 10^4 ±1.3 x 10^3, and 3.3 x 10^3 ± 9.2 x 10^1 CFU/g respectively as shown in Table 4. It was noticed that no considerable increase in the bacterial counts to the 10th day of storage, meanwhile a significant increase ($P<0.05$) was observed starting from the 10th day.

Table 1. Sensory evaluation of *Oreochromis niloticus* samples stored in ice

Sensory Quality parameters	Storage Time (day)								
	0	2	4	6	8	10	12	14	16
1-Appearance	0.00	0.00	0.00	3.00	3.00	3.00	5.00	5.00	6.00
2-Eyes	0.00	2.33	3.00	3.00	3.00	4.00	4.00	4.33	6.00
3-Gills	0.00	0.00	1.00	0.00	2.00	2.00	2.00	4.00	4.00
4-Muscle Color	0.00	0.00	0.00	0.00	0.33	1.00	1.00	1.00	4.00
5-Internal abdominal wall	0.00	0.00	0.67	1.00	1.00	1.67	2.00	2.00	0.67
Quality index	0.00	2.33	4.67	7.00	9.33	11.67	14.00	16.33	18.67

Table 2. Statistical analytical results of organoleptic demerit scores of *Oreochromis niloticus* samples during ice storage

	Chilled storage time (16 day)								
	0	2	4	6	8	10	12	14	16
Min.	0.00	2.00	4.00	7.00	9.00	11.00	14.00	15.00	18.00
Max.	0.00	3.00	5.00	7.00	10.00	12.00	14.00	17.00	19.00
Mean	0.00	2.33	4.67	7.00	9.33	11.67	14.00	16.33	18.67
S.E.	0.00	0.21	0.21	0.00	0.21	0.21	0.00	0.21	0.21

Min. = Minimum. Max. = Maximum. SE = Standard Error

Table 3. Statistical analytical results of pH, TVN and TMB values for fish samples during ice storage

Quality parameters		Chilled storage time (16 day)								
		0	2	4	6	8	10	12	14	16
pH	Min.	5.80	5.83	5.90	5.93	6.10	6.20	6.30	6.35	6.49
	Max.	5.83	5.88	5.93	5.99	6.20	6.29	6.34	6.46	6.52
	Mean	5.82	5.86	5.91	5.96	6.15	6.25	6.32	6.42	6.50
	S.E.	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.01
TVB-N	Min.	8.96	10.50	11.50	13.40	13.66	16.50	19.00	21.50	29.79
	Max.	9.63	11.11	12.60	13.89	15.01	17.02	19.71	23.00	30.46
	Mean	9.38	10.94	12.15	13.56	14.50	16.83	19.32	22.55	30.03
	S.E.	0.11	0.09	0.19	0.08	0.22	0.08	0.11	0.23	0. 14
TMA-N	Min.	2.75	3.25	4.00	4.75	5.30	6.00	6.85	7.50	9.95
	Max.	2.80	3.45	4.30	4.80	5.45	6.20	7.00	7.80	10.10
	Mean	2.78	3.37	4.17	4.78	5.38	6.10	6.93	7.67	10.02
	S.E.	0.01	0.04	0.06	0.01	0.03	0.04	0.03	0.06	0.03

Min. = Minimum. Max. = Maximum. SE = Standard Error
TVB-N= Total volatile basic nitrogen
TMA-N= Trimethylamine nitrogen

The acidic pH, ice chilling and the natural resistance of healthy fish were favorable factors contributed to slow the bacterial growth. Moreover, the alkaline pH, abuse storage temperature and invasion of bacteria to the tissues (from gills through the vascular system or directly form the intestine or skin) were a favorable factors contributed to increase the bacterial counts beyond the 10th day. This concept agrees with EOSQC (2005) which stated that the shelf life of the chilled whole fish zero 0°C not exceed 11 days from catching. A significant high correlation between each of the bacterial counts, pH, TVB-N, TMA-N and sensory characters as evidence by the considerable values of these factors were in agreement with the results recorded by Martinsdottir *et al.* (2001), Elbassuony (2005) and Okeyo *et al*. (2009).

Pseudomonas putida biovar B, *Pseudomonas fluorescens, Shewanella putrefaciens, Aeromonas sobria, Aeromonas caviae* and *Aeromonas allscharophilia* are the phenotypic identified with an incidence of 36.59% (60), 10.98% (18), 9.15% (15), 21.95% (36), 15.24% (25) and 6.10% (10) respectively (Table, 5). These values illustrated that the main microbial flora detected in the examined samples were of *Pseudomonas,* followed by *Shewanella and Aeromonas* species, the main specific spoilage organisms "SSO" as considered by Huss (1995). *Pseudomonas* species, predominating *Shewanella putrefaciens* due to the inhibitory activity of the isolated siderophore producing *Pseudomonas* through the competition inhibition of iron and the antibiotic activity of the siderophore (Henry *et al*., 1991; Gram, 1993). On the other hand, the high incidence of the isolated *Pseudomonas* species may be due to their faster growth rates at chill temperature and the shorter of its lag phase (Drosinos and Board, 1994). A significant increase in hydrogen sulfide producing isolates during the ice storage period, which coincided with rise in pH, TVN-B and total aerobic counts. This regards to the activity of *Pseudomonas* species and *Shewanella putrefaciens* with the aid of *Aeromonas* species. The microbial activities include decomposition of lipids, protein and non-protein nitrogen compound results in undesirable metabolites such as ammonia, indole, TVN-B and other volatile base nitrogen, DMA-B, TMAO-B, TMA-B and H_2S with disagreeable effect end by off odor and bad flavor (Goulas, *et al.,* 2005; Oyelese, 2006). The continuous activity of *Pseudomonas fluorescens* and *Shewanella putrefaciens* during ice storage regards back to the

Table 4. Statistical analytical results of the bacterial counts (CFU/g) recovered from the fish samples during ice storage

		Chilled storage time /day								
		0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
APC	**Min.**	$3.0X10^3$	$5.0X10^3$	$6.5X10^3$	$1.0X10^4$	$1.7X10^4$	$5.0X10^4$	$9.0X10^4$	$1.1X10^5$	$2.0X10^6$
	Max.	$4.0X10^3$	$5.6X10^3$	$7.5X10^3$	$1.2X10^4$	$1.8X10^4$	$6.0X10^4$	$1.0X10^5$	$1.2X10^5$	$2.3X10^6$
	Mean	$3.5X10^3$	$5.4X10^3$	$7.0X10^3$	$1.1X10^4$	$1.7X10^4$	$5.5X10^4$*	$9.5X10^4$ *	$1.2X10^5$*	$2.2X10^6$ *
	S.E.	$1.8X10^2$	$1.2X10^2$	$1.8X10^2$	$3.7X10^2$	$2.8X10^2$	$1.8X10^3$	$1.8X10^3$	$1.8X10^3$	$5.6X10^4$
TPsC	**Min.**	$1.0X10^3$	$2.0X10^3$	$4.0X10^3$	$8.0X10^3$	$1.1X10^4$	$1.3X10^4$	$1.8X10^4$	$9.0X10^4$	$1.0X10^6$
	Max.	$1.2X10^3$	$2.2X10^3$	$5.0X10^3$	$9.5X10^3$	$1.2X10^4$	$1.6X10^4$	$2.0X10^4$	$1.0X10^5$	$1.0X10^6$
	Mean	$1.1X10^3$	$2.1X10^3$	$4.5X10^3$	$8.8X10^3$	$1.2X10^4$	$1.4X10^4$ *	$1.9X10^4$ *	$9.5X10^4$ *	$1.2X10^6$ *
	S.E.	$3.7X10^1$	$3.7X10^1$	$1.8X10^2$	$2.8X10^2$	$1.8X10^2$	$5.6X10^2$	$3.7X10^2$	$1.8X10^3$	$9.2X10^4$
HSPPs	**Min.**	$1.0X10^3$	$2.0X10^3$	$4.0X10^3$	$8.0X10^3$	$1.1X10^4$	$1.3X10^4$	$1.8X10^4$	$9.0X10^4$	$1.0X10^6$
	Max.	$1.2X10^3$	$2.2X10^3$	$5.0X10^3$	$9.5X10^3$	$1.2X10^4$	$1.6X10^4$	$2.0X10^4$	$1.0X10^5$	$1.5X10^6$
	Mean	$7.8X10^2$	$1.4X10^3$	$2.1X10^3$	$3.5X10^3$	$4.5X10^3$	$7.5X10^3$ *	$1.2X10^4$ *	$1.3X10^4$ *	$1.6X10^4$ *
	S.E.	$2.7X10^1$	$3.7X10^1$	$3.7X10^1$	$1.8X10^2$	$1.8X10^2$	$1.8X10^2$	$2.1X10^2$	$2.8X10^2$	$3.7X10^2$
Pseudomonas	**Min.**	$8.5X10^2$	$1.8X10^3$	$3.0X10^3$	$6.0X10^3$	$8.0X10^3$	$1.0X10^4$	$1.2X10^4$	$2.5X10^4$	$3.5X10^4$
	Max.	$1.0X10^3$	$2.0X10^3$	$4.0X10^3$	$8.0X10^3$	$9.0X10^3$	$1.2X10^4$	$1.4X10^4$	$5.0X10^4$	$6.0X10^4$
	Mean	$9.2X10^2$	$1.9X10^3$	$3.5X10^3$	$7.0X10^3$	$8.7X10^3$	$1.1X10^4$ *	$1.3X10^4$ *	$3.0X10^4$ *	$5.5X10^4$*
	S.E.	$2.7X10^1$	$3.7X10^1$	$1.8X10^2$	$3.7X10^2$	$2.1X10^2$	$3.7X10^2$	$3.7X10^2$	$1.8X10^3$	$1.8X10^3$
TPC	**Min.**	$8.0X10^2$	$1.7X10^3$	$3.5X10^3$	$4.4X10^3$	$6.0X10^3$	$9.0X10^3$	$1.1X10^4$	$1.4X10^4$	$2.8X10^4$
	Max.	$9.5X10^2$	$2.3X10^3$	$4.5X10^3$	$5.6X10^3$	$8.0X10^3$	$1.1X10^4$	$1.3X10^4$	$1.6X10^4$	$3.5X10^4$
	Mean	$8.8X10^2$	$2.0X10^3$	$4.0X10^3$	$5.0X10^3$	$7.0X10^3$	$1.0X10^4$ *	$1.2X10^4$ *	$1.5X10^4$ *	$3.1X10^4$ *
	S.E.	$2.8X10^1$	$1.1X10^2$	$1.8X10^2$	$2.2X10^2$	$3.7X10^2$	$3.7X10^2$	$3.7X10^2$	$3.7X10^2$	$1.3X10^3$
TLC	**Min.**	$1.0X10^2$	$2.0X10^2$	$4.5X10^2$	$8.0X10^2$	$1.0X10^3$	$1.4X10^3$	$1.8X10^3$	$2.4X10^3$	$3.0X10^3$
	Max.	$2.0X10^2$	$3.0X10^2$	$5.5X10^2$	$1.0X10^3$	$1.5X10^3$	$1.6X10^3$	$2.2X10^3$	$2.6X10^3$	$3.5X10^3$
	Mean	$1.5X10^2$	$2.5X10^2$	$5.0X10^2$	$9.0X10^2$	$1.2X10^3$	$1.5X10^3$ *	$2.0X10^3$ *	$2.5X10^3$ *	$3.3X10^3$ *
	S.E.	$1.8X10^1$	$1.8X10^1$	$1.8X10^1$	$3.7X10^1$	$9.2X10^1$	$3.6X10^1$	$7.3X10^1$	$3.7X10^1$	$9.2X10^1$

Min. = Minimum. Max. = Maximum. SE= Standard Error. (*) = significant P value if less than 0.05
TAC= Total aerobic counter
TPsC= Total psychrophilic count
HSPPs= Hydrogen sulphite producing psychrophilic
TPC= Total proteolytic count
TLC= Total lypolytic count

Table 5. Incidence of the phenotypic identified bacterial stains recovered from *Oreochromis niloticus* fish samples

Isolates	**Action**	**No.**	**(%)**
Pseudomonas putida biovar *B*	Psychrophilic - Proteolytic	60	36.59
Pseudomonas fluorescens	Psychrophilic - Proteolytic -H_2S producer	18	10.98
Shewanella putrefaciens	Lipolytic - H_2S producer	15	9.15
Aeromonas sobria	Psychrotrophic H_2S producer	36	21.95
Aeromonas caviae	Psychrotrophic – Fish spoiler	25	15.24
Aeromonas allscharophilia	Psychrotrophic – Fish spoiler	10	6.10
Total		164	100

stability of their execrated enzymes (lipolytic and proteolytic). The isolates correlated better with the remaining shelf life than did total viable counts, could be explain that spoilage is more often a result of the production of off odor and flavor caused by specific spoilage organisms, which are only a fraction of the total microflora (Koutsoumanis and Nychas, 1999; Goulas, *et al*., 2005).

Huss (1995) and Yagoub (2009) reported that the monitoring and controlling of fish quality is one of the main goals in fish industry. Therefore, the maintenance of the quality and the extension of fish shelf life by chilling are essentially through the reduction in the growth rate and metabolic activity of spoilage microorganisms. In conclusion, fish should chilled to temperature of zero directly after capture and maintained at this temperature until it reaches the consumers to retards the spoilage and extended their acceptability. Overfilling the storage containers will cause bruising of fish, which better to be avoid. A good time/temperature storage and prevention the cross contamination should maintained during chilling.

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