Pak. j. sci. ind. res., vol. 33, no. 7, July 1990

MICROBIOLOGICAL, CHEMICAL AND SENSORY ASSESSMENT OF POND REARED TILAPIA *OREOCHROMIS MOSSAMBICUS* STORED IN ICE

NOOR-UN-NISA QADRI, MUHAMMAD, ILYAS AND ISMAT MAHMOOD
PCSIR Laboratories Complex, Karachi , Pakistan

(Received January 6, 1990; revised July 25, 1990)

Chemical, sensory and microbiological analyses were carried out on pond reared Tilapia during 21 days of storage in ice (0^0C). At the time of harvest average bacterial count of the pond reared fish was 2.6 x 10^2/g, while pond water contained 6 x 10^3/ml. *Pseudomonas* and *Flavobacterium* were dominant organisms throughout the experiment. The total bacterial count of fish during the first 6 days reached upto 5.85 x 10^3/g, after which there was a constant increase in total bacterial load and at the end of the experiment it reached upto 5.3 x 10^7/g.

Thiobarbituric acid, Trimethyle amine-nitrogen, total volatile nitrogen and pH values during this period increased with the increase in total bacterial count. Proximate analyses of chemical contents were carried out on representative samples of the fish. Sensory results indicated that tilapia used in this trial had a shelf life of 6 days.

Key words: Tilapia, Ice storage, Post-harvest deterioration.

Introduction

Fish and fish products are particularly susceptible to post harvest deterioration. Much information is available about the spoilage mechanism and number of types of micro-organisms associated with marine fish [1-3]. A brief account of microbial flora of Tilapia is given [4,5]. No information is available about the chemical and sensory behaviour of this type of fish which has been studied for the first time by the authors. The object of the study was to determine the shelf life of Tilapia in ice by use of microbial, chemical and sensory assessment. In addition, data is provided on change in the total bacterial counts and type of the bacteria on fresh water pond reared Tilapia during storage in ice (0°C).

Materials and Methods

Pond fish and sample preparation. Tilapia evaluated in this study were reared on artificial feed (28% protein) in a pond. The fish were harvested by drage net in the month of June. Immediately after harvest the fish were iced, weighed, then gutted, cleaned and stored in separate plastic bags in crushed ice within 4 hr in an insulated ice container which was drained and re-iced daily till 21 days.

Sampling. Immediately after sample preparation at 0-day, physical characters and raw sensory attributes were determined. Samples for microbiological and chemical analyses were also taken and kept at -40^0 until analyses completed. Subsequently, ice stored fish were sampled every 3rd day until 21 days. At each sampling time microbiological, chemical and sensory analyses of raw and cooked fish were carried out on 3 randomly taken fish.

Attributes to raw fish. The fish were examined for change in gill colour and odour. Each fish was described by at least 3 persons experienced in fish quality evaluation.

Microbiological analyses. Water samples were collected at three different locations of the pond immediately before harvesting. Fish samples were prepared for plating by cutting surface (2cm^2) from both the outside surface and body cavity using sterile forceps and scalpels. Three fish were sampled in this manner on each sampling day. Using sterile techniques, the samples were cut into smaller parts and blended for two min. Bacterial number of pond water and Tilapia were determined with the spread plate method using Trypitic Soy Agar (TSA, Difco). Appropriate solutions of fish samples and pond water in 0.1 ml quantities were spread evenly on the surface of the prepared agar plates. Plates were incubated at 30^0 for 72 hr Colony Forming Units (CFU) were counted after 3 days of aerobic incubation and the logarithmic mean were calculated. To determine the microbial types, representative colonies appearing on plates were picked and placed on TSA slants. Isolates were identified to generic level according to the Cowan *et.al.*[6] and Bergey's Manual [7].

Chemical analyses. Thiobarbituric acid determination were performed as per Vyncke method [11], and results are expressed as mg melanoaldehyde (MA)/kg tissue. Total volatile nitrogen (TVN) and Trimethylamine nitrogen (TMA-N) were determined [12, 13].

Sensory evaluation. From ice stored fish skinned by hand, a sample (approximately 3cm x 3cm) was removed from anterior dorsal region of each of 3 fish. Samples were placed in individual petri dishes steamed for 20 min. in a water bath and then served in these dishes to the panelist. The sensory evaluation panel consisted of 5 members of the research staff who were experienced in sensory evaluation of fish. The panel rated the samples for odour, flavour, texture, and overall acceptability using a 5 points hedonic

scale (5 = like very much, 4 = like slightly, 3 = neither like nor dislike, 2 = dislike slightly, 1 = dislike very much (Table 1). Mean score for intensity and degree of acceptability were determined for each term [14].

Results and Discussion

Microbiological analyses. Aerobic plate counts (APC) of water taken from the culture ponds averaged 6.00 x 10^3 per ml, while initial counts of Tilapia immediately after harvesting averaged 6.35 x 10^2 per cm (Table 2). All plate counts exhibited an approximately logarithmic increase throughout the trial. Recommendations set by the International Commission on Microbiological Specifications for Foods (ICMSF) [17] were met during the shelf life, in that flesh Aerobic plate counts did not exceed 10^6/g weight in more than 2 - 5 samples and never exceeded 10^7/g. The last sampling time these recommendations were met at the end of the experiment at day 21, when a total count 5.3 x 10^7 per gram had been reached. Throughout the study analyses of variance [15] indicated no significant difference ($P<0.05$) between the counts of the outer surface and body cavity of fish. The distribution of microbial types of water and pond reared Tilapia were almost same except that *Lactobacillus* and *Alcaligence* were absent in water and Bacilli were present in water which were absent in fish. The microbial flora of the pond water from which the fish were harvested contained more than 90 % gram negative and less than 10 % gram positive bacterial with *Flavobacterium* and *Proteus* species predominating (Table 2). The fish immediately after harvesting showed a predominance of gram negative organisms with *Pseudomonas* and *Flavobacterium*, species dominating similar results have previously been observed [4]. In addition to an increase in total count after the fish were held on ice, there was reduction in gram positive bacteria and *Vibrio*, after the 6th day of storage in ice, their count reached to zero being mesophilic in nature. As storage on ice proceeded beyond the first day, the population again shifted to a rapid increase of *Flavobacterium* species, which are psychrotrophic organisms. Until the end of the experiment at day 21, there was constant increase in total bacterial load. At the end of the experiment at day 21, the total count was 5.3 x 10^7 100% of gram negative bacteria. Similar observations have been made earlier [16].

pH measurement. The initial pH taken approximately 4 hr after harvesting was 6.6. During the first 6 days on ice pH of Tilapia decreased to 6.25 after which there was increase reaching a pH of 6.71 at the end of the trial. The increase in pH was probably due to production of basic volatiles compounds by bacteria during the spoilage process (Fig. 2).

Thiobarbituric acid number. The odour and flavour in sensory evaluation of the cooked fish did not coincide with the increase in TBA No., therefore, the increase in TBA No. did not imply loss of acceptability and so was not useful for assessing the end of the shelf life in this experiment.

TABLE 1. CHANGES IN TEXTURE, FLAVOUR AND ODOUR OF COOKED TILAPIA DURING ICE STORAGE.*

Observation	Days in ice							
	0	3	6	9	12	15	18	21
Texture	5.0±0.115	4.5±0.173	4.2±0.115	2.6±0.182	2.0±0.00	1.9±0.182	1.6±0.058	1.0±0.115
Flavour	5.0±0.115	4.3±0.115	3.0±0.183	2.5±0.115	2.0±0.182	1.8±0.182	1.2±0.058	0.8±0.182
Odour	4.5±0.00	4.0±0.00	3.6±0.00	3.0±0.00	2.3±0.00	1.8±0.00	1.2±0.00	0.7±0.00
Gills (raw)	Dark red	Dark red	Light red	Brownish red	Brownish red	Brown	Stale	Rancid

* Each mean is the average of 3 observations.

TABLE 2. PERCENTAGE DISTRIBUTION OF MICROFLORA OF POND WATER AND TILAPIA HELD IN ICE FOR 21 DAYS.

Sample	Aerobic plate count	Pseudo-monas	Flavobac-terium	Vibrio	Alcali-genes	Proteus	Micro-coccus	Strepto-coccus	Lactoba-cillus	Bacillus	Yeast	Fungus
Water	$6.00x10^3$	00.66	77.10	2.90	-	10.04	0.42	0.51	-	8.37	-	Asp
day 0	$6.35x10^2$	24.00	54.70	9.69	3.30	03.14	3.00	0.78	1.58	-	-	-
day 3	$9.65x10^2$	23.50	62.50	12.30	0.15	-	1.45	-	-	0.10	+	Asp
day 6	$5.85x10^3$	17.79	65.40	8.10	0.02	-	0.12	0.01	-	8.56	+	Asp
day 9	$4.59x10^3$	12.84	81.79	5.6	0.02	-	0.02	-	-	-	-	Asp
day 12	$1.10x10^4$	45.43	54.52	-	0.03	-	0.02	-	-	-	-	-
day 15	$7.00x10^5$	30.00	70.00	-	-	-	-	-	-	-	-	-
day 18	$9.10x10^6$	1.10	98.68	-	-	-	-	-	0.22	-	+	-
day 21	$5.30x10^7$	-	100.00	-	-	-	-	-	-	-	-	Asp

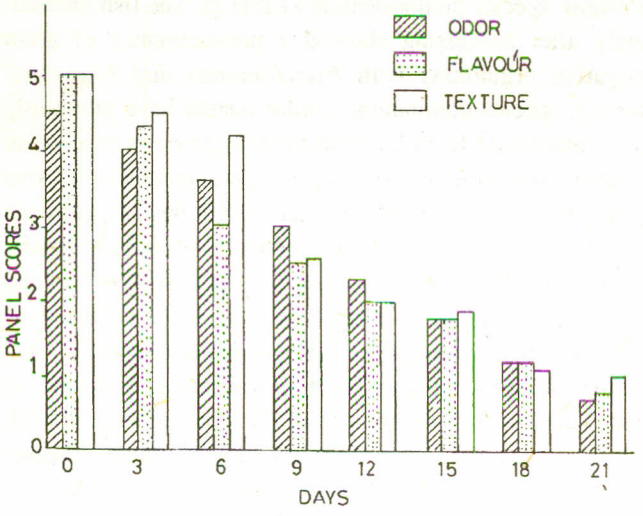

Fig. 1. Sensory panel scores of over all acceptability of cooked Tilapia.

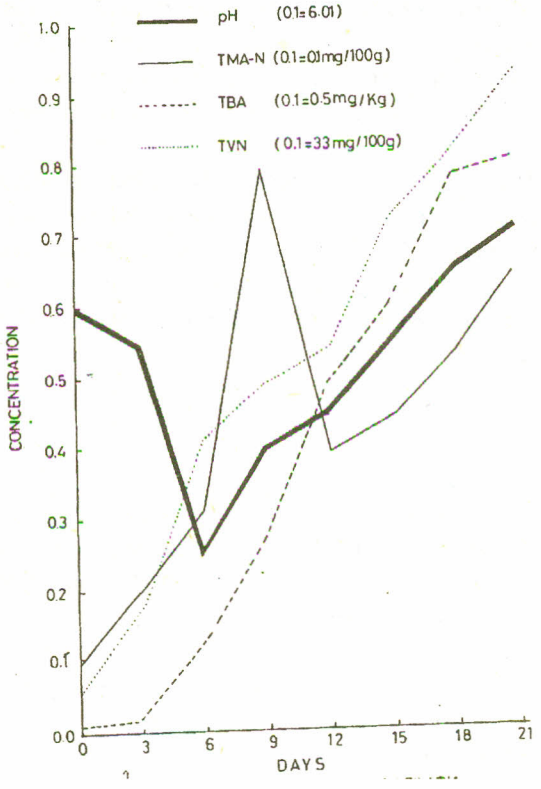

Fig. 2. Ph, TMA-N., TBA and TVN values of Tilapia during storage in ice (0°C).

Trimethylamine and total volatile nitrogen analyses. Changes in TMA levels of Tilapia held on ice are shown in Table 1. The initial value taken approximately 5 hr after harvesting was 0.01. During the first 6 days of storage on ice there was a gradual increase in TMA-N. From 7th to 9th day there was a rapid increase in TMA-N values reaching upto 0.07mg. Thereafter it decreased upto 0.039 mg after that there was again a gradual increase reaching upto 0.065 mg till the end of the experiments. This minimal increase suggests that the measurement of TMA-N is not a good indicator of freshness. The production of volatile nitrogen compounds in Tilapia stored in ice is shown in Fig.2. TVN content at day 0 was 1.91 mg/100 mg indicating fish of high quality. There was a steady increase in the total volatile nitrogen content reaching a level of 31.00 mg/100 g on day 21 of ice storage. This increase was correlated with the APC of 10^6/g at day 21 emphasizing the use of TVN as an indicator of the bacterial spoilage in this study.

SENSORY EVALUATION:

Raw fish. The gills were initially dark red with a thin mucus, but after 4 days were changed to brownish red with thick mucus and a rancid odour (Table 1).

Cooked fish. Fresh fish stored upto 6 days in ice were described by panel as acceptable after which sourness in taste and flavour were detected. This, alongwith an astringent flavour and fibrous texture, was attributed an unfavourable sensory score by the panel. Later, sourness become progressively more intense and texture became dry, soft and sticky. Thus, the Tilapia used in this trial had a shelf life of 6 days in ice (Table 1).

Conclusion

Based on the results of this study, the shelf life of pond reared Tilapia sotred in ice was found to be 6 days, after which fish developed spoilage characteristics which were disliked by the panel.

The microbial counts of fish did not exceed the limits recommended by ICMSF until day 15, well after the shelf life determined by the sensory evaluation. *Vibro* were a small perrcentage of the microflora during the initial state of trial and the disappearance of the same was most likely due to the cold environment of storage. pH was not a good indicator of early storage changes and TBA No. could not be used to determine loss of acceptability or end of shelf life.

Trimethylamine concentration did not significantly increase throughout the period of ice storage and was found to be less useful as an objective measurement of freshness and did not appear to be the all causes for decrease flavour and odour. The significant increase in TVN content of ice-stored fish was correlated with an increase in APC of fish emphasizing the use of TVN as an indicator of shelf life of ice-stored fish. This increase in TVN did not correspond with the slight increase in pH.

The results of the chemical and microbiological analyses indicated that none of them were correlated with texture, odour, flavour, overall acceptability of pond reared Tilapia.

References

1. J.R. Botta and D.H. Shaw, J. Fd. Sci., **41**, 128501288 (1976).
2. M.E. Waters, Mar. Fisheries Review, **44** (11) (1982).
3. T. Chai, C. Chen, A. Rosen and R.E. Levin, Appl. Microbiology, **16**(11), 1739 (1968).
4. G. Acuff, A.L. Izat and G. Finne, J. Fd. Protection, **47**(10), 778 (1984).
5. N.N. Qadri, J. Khalid, Storage Stability of Tilapia in Relation to Dietary Levels of Tochopherol (1988), in press.
6. S.T. Cowan, *Manual for the Identification of Medical Bacteria* (Cambridge University Press, Cambridge 1981).
7. R.S. Breed, E.G.D. Murray and N.R.Smith, *Bergy's Manual of Determinative Bacteriology* (Williams and Wilkens Company, 1975).
8. *Official Methods of Analyses of the Association of Official Analytical Chemist* (AOAC), et. William Horwitz (1975).
9. J. Floch, M. Less and S.G.H. Stanley, J. Biol. Chem., **226**, 497 (1956).
10. P. Vilege, Nz. J. Sc. , **25**, 155 (1982).
11. W. Vyneke, Fette Seifer Anstrichmitted, **72**(12), 1084 (1970).
12. B. Cobb III, I. Alaniz and C.A. Thompson (Jr.) , J. Fd. Sci., **29**, 431 (1973).
13 W.J. Dyer, J. Fish Res. Bd., Canada, **6**, 351 (1954).
14. G.M. Vaisey, H. Moskowitz, J. Solms and H.J. Roth, Sensory Response to Food-4 Workshop, Forster Verlag A.G., Zurich, Switzerland (1977).
15. W.W. Daniel, *Biostatistics* (John Wiley and Sons, USA, 1978).
16. A. Alian, Studies on Fish Preservation in Zambia Food Technology Research Report FTI, Lusaka, `Zambia, NCSR/ TR **38**, 1-8 (1978).
17. I.C.M.F. Sampling Plans for Fish and Fishery Product, *Micro-organisms in Food* (Univ. of Toronto, Toronto, Canada, 1976), Vol. 2.