The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawaii at Manoa Library
and
University of Hawaii Aquaculture Program in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

Ozone Action on Survival and Storage Life of Live and Chilled Tilapia

L. Glatman[1], O. Sachs[2], Y. Khanin[2], V. Drabkin[1] and A. Gelman[1]*

[1] Kimron Veterinary Institute, P.O. Box 12, Bet Dagan 50250, Israel

[2] Dor Aquaculture Experimental Station, Dor, Israel

(Received 5.2.06, Accepted 12.6.06)

Key words: *Oreochromis*, tilapia hybrids, ozone treatment, survival, storage life, temperature, fresh fish

Abstract

The effects of applying ozone treatment to live pond-raised tilapia on the survival of live fish and length of storage of chilled fish were evaluated according to sensory, chemical, physical, and bacteriological tests. Long-term low-level ozonation extended survival and improved the physical state of live fish in a tank and may be feasible for use in fish markets to improve commercial quality of live fish. Ozone treatment of tilapia while still alive prolonged their storage life as chilled tilapia by 12 days (40%) and improved their quality when stored at 0°C but had little effect at 5°C. The improvement probably resulted from an initial reduction and prevention of growth of spoilage bacteria such as *Pseudomonas fluorescens*, *Shewanella putrefaciens*, and *Aeromonas sobria*. At 0°C, *P. fluorescens* was most sensitive to ozone, while *Brevundimonas diminuta* and *Pseudomonas putida* were not. The combination of ozone pretreatment and storage at 0°C appears to be a feasible means of prolonging the storage life of chilled tilapia.

Introduction

Consumers tend to prefer fresh and chilled rather than frozen fish (Ashie et al., 1996). Therefore, the fish-processing industry is actively seeking methods for extending the survival of live fish in fish market tanks and the shelf life and quality of fresh chilled fish. One method that could achieve these objectives involves ozone, a strong antimicrobial agent that is effective against the majority of gram-positive and gram-negative bacteria, molds, yeasts, parasites, and viruses in gaseous and aqueous phases (Moore et al., 2000).

Ozone applications in the food industry are mostly related to the decontamination of product surfaces and water treatment (Hobbs, 1991; Kim et al., 1999). Ozone has been used with mixed success to inactivate contaminant microflora on meat, poultry,

* Corresponding author. Tel.: +972-3-9681724, fax: +972-3-9681753, e-mail: alexander_gelman@hotmail.com

eggs, fish, fruits, vegetables, and dry foods (Kim et al., 1999, 2003). Until now, and in the fish processing industry, ozone has been used predominantly on an experimental basis (Haragushi et al., 1969; Nelson, 1982; Chen et al., 1992). Little has been reported on the use of ozone on aquacultured freshwater fish (Ravesi et al., 1987; Da Silva et al., 1998; Kim et al., 2000).

The purpose of the present study was to evaluate the effects of ozone on the survival and storage life, respectively, of live and chilled pond-raised tilapia. The objectives were to determine the effects of long-term low-level ozonation on fish survival in a tank and the effect of ozone pretreatment on fish shelf life and microflora stored at 0 or 5°C.

Materials and Methods

Fish. Pond-raised tilapia (*Oreochromis niloticus* x *Oreochromis aureus*), averaging 200-250 g, were reared in earthen ponds at the Dor Aquaculture Experimental Station.

Ozone treatment. For the long-term low-level ozone experiment, fish were kept in the tank at a density of approximately 10 kg fish (40-50 fish) per 100 l water for 5 (Experiment 1) or 11 days (Experiment 2), with and without ozonation in conditions similar to those in fish markets. Water circulation was 10% per day. The ozone concentration at entry was 0.1-0.12 mg/l; the residual concentration was 0.02-0.03 mg/l. Oxygen concentration was 8.0-8.5 mg/l and water temperature 23±1.5°C (Experiment 1) or 20±1.5°C (Experiment 2).

In the storage experiment, live fish were treated with 6 ppm ozone in a 100-l tank by means of a G22 ozonizer (Pacific Ozone Technology, Brentwood, California, USA) for 1 h. The ozone concentrations were measured continuously, on-line, with a Dissolved Ozone Monitor, Model A15/64 (Analytical Technology Inc., Collegeville, PA). A ventilation system was used to remove excess ozone. The water temperature ranged 20-23°C.

Packing and transportation. Ozone-treated and untreated (control) live fish were transferred from the Dor Aquaculture Experimental Station to the laboratory at Bet Dagan in sealed plastic bags. The bags were placed in styrofoam boxes with abundant ice placed beneath, on top of, and between the bags. The boxes were immediately sealed to ensure a rapid drop in the activity of the fish. The body temperature of the fish dropped to 4°C and death occurred within approximately 20 min. The time from packing to arrival at the laboratory was 2 h and the temperature of the fish upon arrival was 2°C. The fish that had undergone long-term ozonation were taken for bacteriological and sensory analysis immediately after arrival.

In the storage experiment, fish were individually packed in sealed polyethylene bags. Samples of four ozone-treated and four untreated fish were taken for evaluation on the day of arrival. The bags were stored for 30 days at 0 or 5°C in incubator-refrigerators (Hotpack, Model 352602, Philadelphia, PA; FOC 225I, VELP, Scientifica, Milan, Italy). Samples of four fish were randomly taken for analysis from each treatment after 4, 8, 11, 14, 18, 22, 25, and 30 days of storage.

Sensory analyses. Fresh fish were evaluated by assigning scores to twelve features on a 10-point scale ranging from 10 (best) to 2 (worst; Gelman et al., 2001). The features were slime (texture and odor), eyes (geometry and color), gills (slime, color, and odor), muscles (texture and stiffness), viscera (odor and appearance), and flesh (odor). Four trained experts with no less than 12 years working experience individually assigned scores to each feature. The average scores and standard deviations were calculated. A freshness score above 4 was the threshold for acceptability. A freshness score of 6 or above was required for a high-quality designation (Huss, 1994).

Physical analysis. Electrical resistance was measured with a Torrymeter (GRI Electronics Ltd., Scotland) in torrymetric units. Four measurements were made on different parts of the body of each fish: one in the dorsal section close to the head, two in the middle part of the body, and one near the tail.

Chemical analysis. Total volatile basic nitrogen (TVB-N) was determined with a

Kjeltec System 1026 (Foss Tecator, Sweden). Bases from the weighed samples were distilled in the presence of magnesium oxide. The distillate was collected in a solution of excess boric acid in the presence of indicators (methyl red mixed with bromocresol green) and titrated with 0.01 N sulfuric acid. The TVB-N value was expressed as grams of N per kg fish (AOAC, 1995).

Bacteriological analyses. Total aerobic bacterial counts were done on the surface, in the gills, and in the flesh of the fish. Surface counts were obtained by placing a 5 x 5 cm^2 sterile template on the fish surface, in the middle region along the lateral line, and swabbing the area enclosed by the template. Gill bacterial counts were obtained by swabbing the gills (approximately 1 cm^2), transferring the swabs to 10 ml of sterile peptone (0.1% w/v) as a diluting medium, and shaking the solution well before plating. For flesh samples, the skin was removed aseptically, underlying muscles (10 g) were removed and homogenized in a Stomacher 400 (Model BA6021, Seward Laboratory, UAC House, UK) for 1 min in 90 ml of sterile peptone (0.1 % w/v), a serial tenfold dilution in the same medium was plated on plate count agar (PCA, Difco), and the plates were incubated at 30°C for 48 h.

Each of the 10 dominant bacterial colonies from each PCA was re-streaked onto nutrient agar and stored pending identification. The following tests were used to determine the purified strains: Gram staining, KOH-test, production of oxidase, and growth on appropriate media (MacConkey agar, Baird-Parker agar, *Pseudomonas* agar, and Triple Sugar Iron agar, Difco). Final biochemical identification was carried out with API NE, API 20E, and API Staph (BioMerieux Vitek, Inc., Marcy-l'Etoile, France).

Statistical analysis. Each measurement was carried out on four samples and results are presented as averages±SD. The significance of differences in sensory values and bacterial counts between ozone-treated and control samples were determined by the Student-Newman-Keul test (Sokol and Rohlf, 1995). Differences were considered significant when $p<0.05$.

Results

Long-term low-level ozonation. The physical status of the fish transported from kibbutz aquafarms in a traditional manner (Experiment 1) and fish captured at Dor Aquaculture Experimental Station undergoing minimal stress (Experiment 2) were compared.

In Experiment 1, long-term low-level exposure to ozone had a positive effect on fish survival in a closed system; practically all the fish were alive and healthy after five days in the tank. In the control groups, most of the fish were ill or dead by the third day and only one or two remained alive on the fifth. In the treated tank, the ozonated water remained clear, the fish were active, fish surfaces were intact without traces of mold or injury, eyes were black and shiny, and slime was transparent and elastic. In contrast, water in the control tank was cloudy by the third day and the surfaces of the fish were dull and partially covered by mold and non-elastic slime.

In the ozonated water, total bacteria counts on the fish surfaces and gills rose insignificantly from 8×10^3 to 2.3×10^4 and from 5.6×10^4 to 1.74×10^5 CFU/cm^2, respectively. In contrast, microbial contamination of the fish surfaces and the gills in the control group increased from 1.1×10^4 to 4.4×10^7 and 1.61×10^5 to 2×10^7 CFU/cm^2 by the fifth day ($p<0.05$). In the ozonated water, the microflora became more varied with time but *Shewanella putrefaciens* was not recorded and the water retained its natural smell. In the control tank the initial microbial composition on the fish surface and gills was composed of *Micrococci, Aeromonas* spp. (*A. hydrophila, A. sobria*), with *Pseudomonas* spp. *(P. aeruginosa* and *P. putida)* predominant. By day 5, the *Micrococci* disappeared, the numbers of *Pseudomonas* and *Aeromonas* increased, and *Acinetobacter lwoffii* and *S. putrefaciens* appeared, the latter most probably being the source of an unpleasant smell in the water.

Experiment 2, in which fish were captured at Dor Aquaculture Experimental Station and transferred to the tank carefully to minimize stress, differed in length from Experiment 1. By day 11, only 26.4% of the fish in the ozonated water were dead whereas 92.4% of

the control fish were dead (Fig. 1). The total bacterial counts and microflora composition were similar in the control and ozonated fish. As the ammonium (NH_4^+) concentration was 6.3 times higher in the control tank than in the treated tank, it could be proposed that the ozone destroyed the ammonium and thus improved water quality.

Sensory properties of stored fish. Ozone treatment of live tilapia prolonged their subsequent shelf life by 12 days when stored at 0°C (Table 1). The control group maintained high quality ratings (6 points) up to 18 days while the ozone-treated fish maintained this quality to 30 days. At 5°C, the control fish maintained the high quality level until day 8 and the ozone-treated fish until day 11, i.e., the shelf life was prolonged by three days in the treated fish. The differences were statistically significant (p<0.05).

Physical analysis of stored fish. At 0°C, the Torrymeter readings dropped faster in the ozone-treated group than in the control during the first seven days and more slowly in the ozone-treated group thereafter (Gelman et al., 2005). The differences between the control and treated groups were significant and persisted throughout the storage period. At 5°C, the readings dropped more rapidly, with no differences between the untreated and ozone-treated groups.

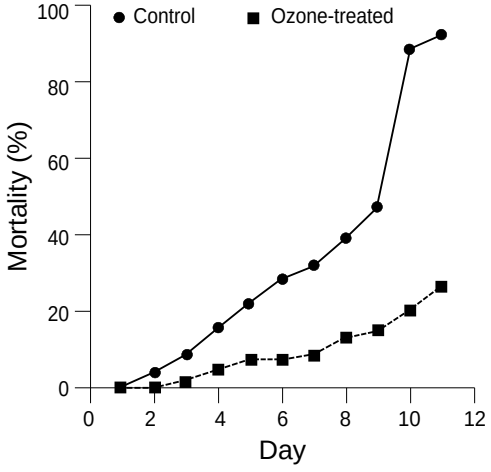

Fig. 1. Effect of long-term low-level ozone treatment on tilapia survival.

Chemical analysis of stored fish. At 0°C, the TVB-N rose steadily during storage but was significantly lower in the ozone-treated fish (Gelman et al., 2005). TVB-N did not reach 0.3 g/kg in the ozone-treated fish, but exceeded this level in the control. The difference between the groups began on day 14 and persisted throughout the storage period of 30 days. At 5°C, the TVB-N rose rapidly in both the control and the ozonized fish, with no significant differences, and exceeded the acceptable level of 0.3 g/kg by day 14.

Bacteria on surfaces of stored fish. At 0°C, the total bacteria counts in the control fish rose continuously and reached around 10^5 CFU/cm^2 by day 14 (Gelman et al., 2005). The counts in the ozone-treated fish rose more slowly and reached around 10^5 CFU/cm^2 by the end of the storage period. At 5°C, the total counts rose rapidly in both groups, exceeding the acceptable level of 10^5 (ICMSF, 1986) on day 8 and reaching around 10^9 CFU/cm^2 by day 14.

Bacteria in muscles of stored fish. At 0°C, the total counts in the control group rose after 20 days and reached about 10^4 CFU/g by the end of storage (Gelman et al., 2005). The ozone-treated fish remained very lightly contaminated (below the registered level 10^2 CFU/g) until the end of storage. At 5°C, the total counts in both groups increased by day 8 and reached 10^5 CFU/g by day 14.

Microbial species composition. The initial background population comprised mostly *Micrococci, Aeromonas* spp., *Enterobacteriaceae*, *Acinetobacter*, and *Pseudomonas* spp.

Microbial composition of fish stored at 0°C. Microflora on the fish surface in the control group disappeared in the following order: *Citrobacter freundii, A. lwoffii*, *Micrococci,* and *A. sobria* (Table 2). They were replaced by *Brevundimonas diminuta* (formerly, *Pseudomonas diminuta*), *P. putida*, and the dominant *Pseudomonas fluorescens* that reached 3.3 x 10^7 CFU/cm^2 by day 30. In the ozonated group, *C. freundii* and *A. lwoffii* were eliminated as early as day 4 and *Micrococci* and *A.*

Table 1. Sensory evaluation of tilapia stored at 0 or 5°C, with and without ozone pretreatment (sensory scores±SD)*.

	Day of storage							
	1	*4*	*8*	*11*	*14*	*18*	*22*	*25*	*30*
Control 0°C	10.0±0.00	9.98±0.09	8.73±0.49	7.25±0.45	7.20±0.80	6.20±0.50	5.30±0.80	5.10±0.70	4.10±0.80
Pretreatment 0°C	10.0±0.00	9.89±0.29	9.48±0.50	8.63±0.52	8.10±0.70	7.40±0.80	6.60±0.80	6.70± 0.50	6.20±0.60
Control 5°C	10.0±0.00	9.94±0.20	6.38±1.10	4.67±0.94	4.50±2.20	-	-	-	-
Pretreatment 5°C	10.0±0.00	9.03±1.03	7.53±1.03	6.39±0.86	5.10±1.10	-	-	-	-

* Sensory scores for twelve features on a 10-point scale from 10 (best) to 2 (worst).

sobria were no longer detected by day 25. After this, *B. diminuta* and *P. putida* dominated.

Contamination in the muscles of the control group was below the detectable level until day 22 (Table 3). On day 22, the same *Pseudomona*s spp. and *B. diminuta* appeared; *B. diminuta* dominated but did not exceed approximately 10^3 CFU/g. By day 30, it was mostly replaced by *P. fluorescens* and *P. putida*. Contamination in the muscles of the ozonated fish remained below the detectable level (<10 CFU/g).

Microbial composition of fish stored at 5°C. By day 8, *Micrococci, A. lwoffii*, and *C. freundii* were no longer detected and *Pseudomonas fluorescens* dominated the surfaces of control fish (Table 4). By day 14, when the fish deteriorated, *P. fluorescens* reached 3.2 x 10^8 CFU/cm^2 and *S. putrefaciens* reached 2 x 10^7 CFU/cm^2. On surfaces of ozone pretreated fish, *Micrococci, A. lwoffii,* and *C. freundii* were undetectable as early as day 4, *A. sobria* rose and reached 3.1 x 10^8 CFU/cm^2 by the end of storage, and *P. fluorescens* was not recorded.

Contamination of fish muscles was not detected in either group until day 8. In the control group, *P. fluorescens* dominated while in the muscles of the ozonated fish *A. sobria* dominated (Table 5).

Discussion

Keeping live fish in water with 0.1 ppm ozone prolonged the survival and improved the physical condition of tilapia. In Experiment 2, survival of fish that had undergone minimal stress was prolonged. Thus, low-level long-term ozonation of freshwater fish together with a ventilation system could be used in fish shops to extend the marketing period and improve the quality. Decreasing stress to fish during harvest and transport is also very important. The beneficial effects of using low-level ozone in an aquaculture water system have been reported by Sutterlin et al. (1984) and Restraino et al. (1995). The extension of the shelf life of perishable foods by using ozone to reduce microbial activity has been reported by Rice et al. (1982) and Dondo et al.

Table 2. Bacterial count (log CFU/cm^2) and distribution (%) of microorganisms isolated from surfaces of tilapia stored at 0°C.

	Day of storage																	
	1		*4*		*8*		*11*		*14*		*18*		*22*		*25*		*30*	
Species	*Count*	*%*	*Count*	*%*	*Count*	*%*	*Count*	*%*	*Count*	*%*	*Count*	*%*	*Count*	*%*	*Count*	*%*	*Count*	*%*
Control (no ozone pretreatment)																		
Micrococcus	1.32	15	2.91	30	3.83	20	3.63	10	-	-	3.91	9	-	-	-	-	-	-
Citrobacter freundii	1.49	22	2.43	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acinetobacter lwoffii	1.32	15	2.63	16	3.53	10	-	-	-	-	-	-	-	-	-	-	-	-
Aeromonas sobria	1.69	35	3.00	38	3.83	20	3.63	10	3.60	4	2.95	1	-	-	-	-	-	-
Brevundimonas diminuta	-	-	-	-	4.08	36	4.41	60	4.39	25	4.48	33	6.38	22	6.95	25	6.94	15
Pseudomonas putida	-	-	-	-	-	-	-	-	-	-	-	-	6.38	22	6.56	10	7.40	45
P. fluorescens	-	-	-	-	3.23	5	3.63	10	4.81	65	4.70	55	6.74	50	7.34	60	7.52	57
Shewanella putrefaciens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Other species	1.25	13	2.20	6	3.48	3	3.63	5	3.78	5	3.25	2	5.69	4.5	6.25	5	6.23	3
Ozone pretreatment																		
Micrococcus	1.0	5	-	-	1.30	10	2.73	15	1.76	15	2.23	10	3.46	3	-	-	-	-
C. freundii	1.74	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. lwoffii	1.62	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. sobria	1.89	37	-	-	1.30	10	2.76	5	-	-	-	-	2.98	1	-	-	-	-
B. diminuta	1.00	5	-	-	2.15	70	3.43	70	2.43	70	3.15	80	4.89	80	5.20	85	4.93	40
P. putida	-	-	-	-	-	-	-	-	1.58	10	1.95	5	3.98	10	4.28	10	5.10	60
P. fluorescens	-	-	-	-	-	-	2.76	5	-	-	1.86	4	3.28	2	-	-	-	-
S. putrefaciens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Other species	1.15	7	-	-	1.30	10	2.76	5	1.28	5	1.26	-	3.58	4	3.98	5	-	-

Dash indicates below detectable level (1.00).

Table 3. Bacterial count (log CFU/g) and distribution (%) of microorganisms isolated from muscles of tilapia that were not pretreated with ozone (control) and were stored at 0°C*.

	Day of storage					
	22		*25*		*30*	
Species	*Count*	*%*	*Count*	*%*	*Count*	*%*
Micrococcus	-	-	-	-	-	-
C. freundii	-	-	-	-	-	-
A. lwoffii	-	-	-	-	-	-
A. sobria	-	-	-	-	-	-
B. diminuta	3.88	80	3.00	12	3.30	25
P. putida	2.88	8	3.32	25	3.23	20
P. fluorescens	3.04	12	3.73	63	3.65	55
S. putrefaciens	-	-	-	-	-	-
Other species	-	-	-	-	-	-

* Microorganisms were below detectable level (1.0) from day 1 through day 18 in the control fish and throughout the storage the ozone pretreated fish. Therefore, they do not appear in this table.

(1992). Similar findings were reported with marine species and ozonated ice (Nelson, 1982) or ozone in controlled atmospheres (Dondo et al., 1992).

Sensory analysis showed that ozone pretreatment of live tilapia prolonged their storage life to 30 days and improved their quality when stored at 0°C. Pretreatment had little effect on tilapia stored at 5°C. Total bacteria counts on the surfaces of pretreated fish were 2-3 log CFU/cm^2 less than in the control fish when stored at 0°C although no differences existed at 5°C. Muscle contamination in ozone-treated fish remained below the detected level until day 30 but only until day 22 in control fish at 0°C. In contrast, the shelf life of catfish fillets was slightly lengthened in storage at 4°C after 10-ppm ozone pretreatment (Kim et al., 2000) and gaseous ozone had a bactericidal effect on five species of fish bacteria inoculated on agar surfaces but much less effect on surface microflora and little effect on muscle microflora of fresh scad stored in ice (Da Silva et al., 1998). The differences between different results could be attributed to differences in experimental design (storage temperature, ozone dosage, exposure time, gaseous or aqueous phases of ozone, etc.). Our storage experiments demonstrate the importance of maintaining a precise temperature with a maximum close to 0°C to maintain the preservative activity of ozone, perhaps accounting for the stronger effect obtained in our study than in those conducted 4°C (Dondo et al., 1992; Kim et al., 2000) or 0±3°C (Da Silva et al., 1998).

The microbial composition on the fish surfaces and muscles was affected by temperature and ozone pretreatment. At 0°C, the initial microflora in pretreated and control fish was gradually replaced by representatives of *Brevundimonas* and *Pseudomonas* spp. Thereafter, the main difference between the two groups was the rapid growth of *P. fluorescens* on the surface of the untreated fish. It

Table 4. Bacterial count (log CFU/g) and distribution (%) of microorganisms isolated from surfaces of tilapia stored at 5°C.

	Day of storage									
	1		*4*		*8*		*11*		*14*	
Species	*Count*	*%*	*Count*	*%*	*Count*	*%*	*Count*	*%*	*Count*	*%*
Control (no ozone pretreatment)										
Micrococcus	1.32	15	-	-	-	-	-	-	-	-
C. freundii	1.49	22	2.32	10	-	-	-	-	-	
A. lwoffii	1.32	15	2.78	29	-	-	-	-	-	-
A. sobria	1.69	35	3.72	25	5.50	20	6.67	6	7.60	10
B. diminuta	-	-	-	-	-	-	-	-	-	-
P. putida	-	-	-	-	4.91	5	-	-	-	-
P. fluorescens	-	-	3.78	29	6.04	70	7.82	85	8.50	80
S. putrefaciens	-	-	2.02	5	4.91	2	6.59	5	7.32	5
Other species	1.25	13	2.62	2	4.68	3	6.49	4	7.32	5
Ozone pretreatment										
Micrococcus	1.00	5	-	-	-	-	-	-	-	-
C. freundii	1.74	26	-	-	-	-	-	-	-	-
A. lwoffii	1.62	20	-	-	-	-	-	-	-	-
A. sobria	1.89	37	4.40	68	5.72	80	7.69	70	8.50	61
B. diminuta	1.00	5	-	-	-	4	-	-	7.72	10
P. putida	-	-	3.75	15	4.86	-	6.79	9	7.42	5
P. fluorescens	-	-	-	-	-	-	-	-	-	-
S. putrefaciens	-	-	3.65	12	4.94	16	7.08	18	8.00	20
Other species	1.15	7	3.28	5	4.56	-	6.32	3	7.32	4

is likely that this strain caused the fish deterioration. In ozonated fish, *B. diminuta* and *P. putida* came to dominate, indicating that *P. fluorescens* was the most ozone-sensitive species in our experiment.

At 5°C, *P. fluorescens* tended to dominate in untreated fish, although *S. putrefaciens* and *A. sobria* were also detected. In the ozone-pretreated fish, however, *A. sobria* dominated, accompanied by *S. putrefaciens, B. diminuta,* and *P. putida.* The deterioration in ozone-treated fish was slightly slower than in the control fish but, by day 14, the fish of both groups deteriorated. At this temperature, *A. sobria* appeared to be more resistant to ozone than *B. diminuta* and *P. putida.* Ozone pretreatment and storage at 5°C did not reduce the growth of *S. putrefaciens* and *A. sobria*, whereas storage at 0°C, with or without ozone pretreatment, prevented their growth. Thus, only the combination of ozone pretreatment and storage at 0°C reduces or prevents the growth of the spoilage bacteria *S. putrefaciens, P. fluorescens,* and *A. sobria,* prolonging the tilapia storage time.

Conclusions

Long-term, low-level ozonation of live fish in a tank extended their survival and improved their physical condition. This intervention may

Table 5. Bacterial count (log CFU/g) and distribution (%) of microorganisms isolated from muscles of tilapia stored at 5°C.

	Day of storage					
	8		*11*		*14*	
Species	*Count*	*%*	*Count*	*%*	*Count*	*%*
Control (no ozone pretreatment)						
Micrococcus	-	-	-	-	-	-
C. freundii	-	-	-	-	-	-
A. lwoffii	-	-	-	-	-	-
A. sobria	1.41	32	2.76	11	2.89	9
B. diminuta	-	-	-	-	-	-
P. putida	1.32	25	2.68	9	1.94	1
P. fluorescens	1.53	43	3.62	80	3.89	90
S. putrefaciens	-	-	-	-	-	-
Other species	-	-	-	-	-	-
Ozone pretreatment						
Micrococcus	-	-	-	-	-	-
C. freundii	-	-	-	-	-	-
A. lwoffii	-	-	-	-	-	-
A. sobria	1.81	80	3.49	85	3.98	86
B. diminuta	-	-	2.27	5	2.34	2
P. putida	-	-	-	-	2.82	6
P. fluorescens	-	-	-	-	-	-
S. putrefaciens	1.2	20	2.57	10	2.82	6
Other species	-	-	-	-	-	-

be a feasible means for improving the commercial quality of fish in fish shop tanks. This effect may have been the result of the reduction of bacterial contamination as well as the decrease of ammonium concentration in the ozonated water.

Ozone pretreatment of tilapia while live prolonged the storage life by 12 days (40%) of chilled fish and improved quality characteristics during 30 days of storage at 0°C, but had less effect when stored at 5°C. The beneficial effect was probably the result of the reduction or prevention of the growth of spoilage bacteria such as *P. fluorescens*, *S. putrefaciens*, and *A. sobria*. The combination of ozone pretreatment and storage at 0°C appears to be a feasible means of prolonging storage life.

Ozone sensitivity appeared to differ among bacterial species. In storage at 0°C, *P. fluorescens*, which causes fish spoilage, appeared to be more ozone sensitive than *B. diminuta* and *P. putida*.

Acknowledgements

The study was supported by a grant from the Ministry of Agriculture and Rural Development, no. 863-0024-02: Ozone Treatment in Live and Chilled Fish Marketing Systems. The skillful assistance of K. Chechik and I. Gabay are gratefully acknowledged.

References

AOAC, 1995. *Officials Methods of Analysis. Automated Method*, 940.25 16th ed. Ch. 35:6.

Ashie I.N., Smith J.P. and B.K. Simpson, 1996. Spoilage and shelf life extension of fresh fish and shellfish. *Crit. Rev. Food Sci. Nutr.,* 36:87-121.

Chen H.C., Huang S.H., Moody M.W. and S.T. Jiang, 1992. Bactericidal and mutagenic effects of ozone on shrimps (*Penaeus monodon*) meat. *J. Food Sci.*, 57:923-927.

Da Silva M.V., Gibbs P.A. and R.M. Kirby, 1998. Sensorial and microbial effects of gaseous ozone on fresh scad (*Trachurus trachurus*). *J. Appl. Microbiol.*, 84:802-810.

Dondo A., Nachtman C., Doglione L., Rosso A. and A. Genetti, 1992. Foods: their preservation by combined use of refrigeration and ozone. *Ing. Aliment. Conserve Anim.*, 8:16-25.

Gelman A., Glatman L., Drabkin V. and S. Harpaz, 2001. Effect of storage temperature and preservative treatment on shelf life of the pond raised freshwater fish silver perch (*Bidyanus bidyanus*). *J. Food Prot.*, 64:1584-1591.

Gelman A., Sahs O., Khanin Y., Drabkin V. and L. Glatman, 2005. Effect of ozone pretreatment on fish storage life at low temperatures. *J. Food Prot.*, 68:778-784.

Haragushi T., Simudu U. and K. Aiso, 1969. Preserving effect of ozone on fish. *Bull. Jpn. Soc. Sci. Fish*, 35:915-919.

Hobbs G., 1991, Fish: microbiological spoilage and safety. *Food Sci. Technol. Today* 5:166-173.

Huss H.H. 1994, Assurance of seafood quality. *FAO Fish Tech. Pap.* 334. Rome, FAO. 169 pp.

ICMSF, 1986, *Microorganisms in Foods 2: Sampling for Microbiological Analysis*, 2nd ed. International Commission on Microbiological Specification for Foods, Univ. Toronto Press, Toronto, Canada.

Kim J.G., Yousef A.E. and S. Dave, 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. *J. Food Prot.,* 62:1071-1087.

Kim T.J., Silva J.L., Chamul R.S. and T.C. Chen, 2000. Influence of ozone, hydrogen peroxide, or salt on microbial profile, TBARS and color of channel catfish fillets. *J. Food Sci*., 65:1210-1213.

Kim J.G., Yousef A.E. and M.A. Khadre, 2003. Ozone and its current and future application in the food industry. *Adv. Food Nutr. Res*., 45:167-218.

Moore G., Griffith C. and A. Peters, 2000. Bactericidal properties of ozone and its potential application as a terminal disinfectant. *J. Food Prot.,* 68:1100-1106.

Nelson W., 1982. The use of ozonated ice to extend the shelf life of fresh Alaskan fish. Report submitted to Alaskan Department of Commercial Economic Development Office, Commercial Fish Development, Anchorage.

Ravesi E.M., Licciardello J.J. and L.D. Racicot, 1987. Ozone treatment of fresh Atlantic cod, *Gadus morhua. Mar. Fish Rev*., 49:37-42.

Restraino L.E., Frampton W., Hemphill J.B. and P. Palnikar, 1995. Efficacy of ozonated water against various food-related microorganisms. *Appl. Environ. Microbiol.,* 9:3471-3475.

Rice R.G., Farguhar J.W. and L.J. Bollyky, 1982. Review of the applications of ozone for increasing storage times of perishable foods. *Ozone Sci. Eng.,* 4:147-163.

Sokol R.R. and F.J. Rohlf, 1995. Single-classification analysis of variance. pp. 207-271. In: *Biometry*. W.H. Freeman and Co., New York.

Sutterlin A.M., Couturier C.Y. and T. Devereaux, 1984. A recirculation system using ozone for the culture of Atlantic salmon. *Prog. Fish Cult*., 46:239-244.