

Sensorial and microbial effects of gaseous ozone on fresh scad (*Trachurus trachurus*)

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M.V. DA SILVA, P.A. GIBBS AND R.M. KIRBY. 1998. The bactericidal activity of gaseous ozone was investigated using a commercial ozone generator. Five species of fish bacteria, *Pseudomonas putida*, *Shewanella putrefaciens*, *Brochothrix thermosphacta*, *Enterobacter* sp. and *Lactobacillus plantarum*, were inoculated on agar surfaces and exposed to different ozonation times in a gas chamber. Results showed ozone in relatively low concentrations ($<0.27 \times 10^{-3} \text{ g l}^{-1}$) was an effective bactericide of vegetative cells of the five fish bacteria. The age of the cell culture was shown to influence the cell response following exposure. Survival rate was not linearly related to ozonation time, but exhibited biphasic death over an extended period. Similar bactericidal effects were observed on fish skin treated with ozone daily in the laboratory, with decreases of $1.0 \log \text{ cfu cm}^{-2}$ for the micro-organisms studied. Whole fish treated daily in the laboratory using a commercial ozone generator showed improved scores for sensory analyses compared with the controls. The results were statistically significant. Fish treated on board ships were also analysed for microbiological and sensory changes. Controls were obtained from a similar vessel without the ozone facility in the hold. Similar trends to those recorded in the laboratory for the microbiological and sensory results on ozonated fish were observed.

INTRODUCTION

Ozone has been used as a disinfectant for drinking water for many years. The strong oxidizing nature of ozone has made it a useful agent for the inactivation of bacteria, fungi and viruses (Broadwater *et al.* 1973; Foegeding 1985; Ishizaki *et al.* 1986). It has been reported that sensitivity of micro-organisms to ozone is affected by several factors including humidity, temperature, pH and the presence of organic matter (Hoigné and Bader 1975). Ozone first attacks the bacterial membrane at the glycoprotein, glycolipids, or at certain amino acids such as tryptophan. Ozone also acts on the sulphhydryl groups resulting in disruption of the normal cell. Bacterial death is rapid and often attributed to changes in cell permeability followed by lysis. Ozone was effective against Gram-positive (including spore-formers) and Gram-negative bacteria (Greene *et al.* 1993).

The extension of the shelf-life of perishable foods in gen-

eral by reducing microbial activity using ozone has been reported by Rice *et al.* (1982). The use of ozone as a superficial disinfectant of meat surfaces (Sheldon and Brown 1986; Greer and Jones 1989) and for the preservation of shrimps (Chen *et al.* 1992) has been reported. Interest in the use of ozone for fish preservation, by incorporation in ice, rinse water or in chilling seawater, has been reported. Nelson (1982) reported that the fresh quality of ozone-iced Pacific salmon could be maintained for up to 6 days, although Dewitt (1984) found a possible extension of only 1–2 days in the chill storage life of Gulf of Mexico shrimp with the use of ozonated ice. Ravesi *et al.* (1987) reported that the chill storage life of fresh Atlantic cod was not extended by ozone treatments.

As ozone is a bactericidal agent, it is hypothesized that its beneficial effect on fish shelf-life is due to a reduction in the microbial activity responsible for the loss of quality which appears towards the end of shelf-life (Hobbs 1991). No information, however, is available regarding the effect of ozone on bacteria associated with fish spoilage. Consequently, the aim of this paper is to evaluate the effect of ozone on a variety of

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bacteria which have been linked at one time or another to fish spoilage. Pseudomonadaceae, including *Shewanella putrefaciens*, are generally reported to be important in the process of fish spoilage (Dalgaard 1995). Lactic acid bacteria, including *Brochothrix thermosphacta*, have been reported to be involved in the spoilage of fish stored under modified atmosphere packaging (MAP) (Tassou *et al.* 1995). An important step in the ozone process has been taken with the installation of ozone generating equipment on commercial fishing vessels (personal communication).

The installation of ozone generating equipment on board a commercial fishing vessel permitted analysis of the practical implications of the technology and attempts to correlate improvements in microbial quality with subsequent improvements in perceived commercial quality, reflected as always in the commercial world, by increased value of the product.

MATERIALS AND METHODS

Micro-organisms

The following five bacterial species were obtained from the Culture Collection of AAIRProject CT 94-1946 maintained by the Department of Food Microbiology (Leatherhead Food RA, UK): *Brochothrix thermosphacta* S344, *Enterobacter* sp. S098, *Pseudomonas putida* S117, *Lactobacillus plantarum* S223 and *Shewanella putrefaciens* S184.

Fish samples

Fish were taken directly from the fishing vessel after 2 days of storage on ice. Ozonated fish with 3 days of storage on ice were taken from a fishing vessel equipped with an ozone generator in the bulk ice storage room.

Ozone generation and measurements

For laboratory studies, ozone was produced by a domestic model PR1 (TRIOZON, Spain), using atmospheric air as the source of oxygen. The ozonated air produced at a constant flow rate by the apparatus was passed by a silicone tube to a pump, and then to two plastic desiccator chambers of equal size.

The ozone in the air flow produced by the apparatus ($0.328 \times 10^{-3} \text{ g l}^{-1}$) was measured experimentally by the iodometric method (APHA 1975) and modified by passing the ozone in the air through a gas diffuser. It was then collected directly in the reaction flask. The triiodine ion liberated in 400 ml of buffered iodide solution (2%) and acidified with H_2SO_4 (0.5 mol l^{-1}) was measured by titration with thio-sulphate solution ($0.0005 \text{ mol l}^{-1}$).

The ozone concentration in the air flow inside the desiccators was calculated by a mass balance, based on the total

volume of the desiccator (7 l), the air flow (0.76 l min^{-1}) and the ozone machine production ($0.328 \times 10^{-3} \text{ g l}^{-1}$). It was assumed that the ozone concentration inside the desiccators follows a first order differential equation, as follows:

$$V_t \times dC/dt = -v \times (C - C_o)$$

where V_t = total volume (l), v = air flow (l min^{-1}), t = time (min), C = ozone concentration (g l^{-1}) and C_o = ozone machine production (g l^{-1}).

Integrating the last equation, from $t = 0$ to t , $(C_o - C)/C_o = \exp.(-v \times t/V_t)$ gives the theoretical curve of ozone concentration inside the desiccators.

Ozonation chamber

The six bacterial cultures were held under conditions of constant high relative humidity (100%) and low temperature ($0 \pm 2 \text{ }^\circ\text{C}$) by placing ice inside and outside the two chambers.

The inoculated plates were introduced into the two chambers and then exposed to gaseous ozone for a chosen time. The ozonated air was pumped at a constant flow rate (0.76 l min^{-1}) into the chambers and the different ozone concentrations in the desiccators were obtained by controlling the time of exposure. The same procedure was used to treat fish in the laboratory.

Similar conditions were used on board to maintain fish in a cold condition ($0 \pm 3 \text{ }^\circ\text{C}$) and in an ozone atmosphere. Fish were stored in boxes with ice and an industrial ozone machines using air as the source of oxygen at a continuous flow, was used to produce ozone ($0.25 \times 10^{-3} \text{ g l}^{-1}$).

Experimental design

Fish samples. In a laboratory pilot study, whole fish were divided into three groups of 10: (1) fish treated with ice (control); (2) fish submitted to one initial ozone treatment (60 min) (treatment 1); and (3) fish submitted to one initial ozone treatment (60 min) and daily exposure (30 min) (treatment 2). Treatment 3 comprised 10 whole fish stored in the ship's bulk room under ozone atmosphere conditions and treated daily (30 min). All the above groups were stored in ice after treatment for a total of 10 d.

Sensory analysis according to the European Union (EU) grading scheme (Anon. 1989) and microbiological examination of muscle and skin by total viable count were performed for all groups. Analyses of obligate psychrophiles, Enterobacteriaceae, Pseudomonadaceae, H_2S -positive bacteria and lactic acid bacteria for groups treated during storage, and thiobarbituric acid (TBA) and pH values, were conducted every 2 days. The experiment was performed twice. The data were analysed by ANOVA using the Statview (Abacus Concepts, Berkeley, CA, USA) with the storage time as the

independent variable. Results were considered significant if the calculated *P* value was less than 0.05.

Pure cultures. For each culture, cell suspensions were delivered onto each of two pre-dried plates of Nutrient agar (N.a.) (Lab M, Bury, UK) 9 cm in diameter. In order to study the effect of culture age on ozone sensitivity, plates were pre-conditioned as follows: (1) immediately exposed to ozone; (2) incubated at 20 °C for 4 h before exposure; or (3) incubated at 20 °C for 10 h before exposure. One plate was exposed to ozone for a designated time (15, 30, 45, 60 or 90 min), a duplicate being placed in an ozone-free environment (zero ozonation time). The samples were removed at each sampling time. All the plates were incubated at 20 °C for 24 h. Results are the mean of duplicate experiments.

Growth curves were constructed using absorbance measurement at 600 nm in Nutrient broth (N.b.) (Lab M) to estimate the growth of each bacterial culture at 20 °C in order to standardize the relative culture age on plates before ozone exposure.

Sensory analysis

For sensory analyses, each fish was evaluated by a three-member trained panel using the following characteristics: (1) general external appearance of the fish; (2) condition of the eyes; (3) appearance and odour of the gills; (4) condition of the muscle resistance to pressure and adherence to the skeletal bones; (5) appearance and odour of the abdominal cavity; and (6) colour and odour of the muscle. Samples were rated on a scale from 0 (inedible) to 3 (excellent). When the average score of any of the attributes decreased to a value of 1, the fish was considered unacceptable.

Microbiological analyses

Fish samples. Samples (5 g) of muscle were homogenized in 45 ml of sterile Ringer solution (Lab M) for 2 min. Sections of skin (10 cm²) were swabbed with sterile Ringer solution (Lab M). Several appropriate dilutions for each sample were made in sterile Ringer solution. All counts are the result of duplicate plating using the Drop Count Technique (Miles and Misra 1938) on the agar indicated. Viable counts were made in the following agars under the incubation conditions indicated: total viable count (48 h at 20 °C) in N.a. (Merck, Frankfurt, Germany); obligate psychrophiles (96 h at 10 °C) in N.a. (Merck); Enterobacteriaceae (24 h at 37 °C) in Violet Red Bile Glucose Agar (VRBGA) (Merck); Pseudomonadaceae (48 h at 20 °C) in Pseudomonas agar base supplemented with CFC (Lab M); bacteria H₂S positive (96 h at 20 °C) in Iron agar (Gram *et al.* 1987); and lactic acid bacteria (5 d at 30 °C) in De Man, Rogosa, Sharp (MRS) (Lab M).

pH was determined by blending 5 g samples of fish muscle with 45 ml of sterile Ringer solution for 2 min. This was then measured with a pH meter (Crison Instrument, South Africa).

Pure cultures. For each bacterial culture, decimal dilutions were made to 10⁻⁷ in sterile Ringer solution. Each plate was divided into four equal spaces for the chosen dilutions (10⁰, 10⁻², 10⁻⁴ and 10⁻⁶). Two separate 20 µl aliquots of the cell suspensions were then delivered onto each of two pre-dried plates of N.a. (Lab M). Growth curves were constructed in N.b. (Lab M) to establish the standard growth curve of each culture at 20 °C. The absorbance of each culture was determined at 600 nm every 15 min during the 10 h incubation period. The cocktail culture was prepared from the pure cultures by mixing together 2 ml of each.

2-Thiobarbituric acid measurement

Oxidative changes in fish muscle treated and not treated with ozone were analysed using the TBA test (Anon. 1990). The TBA values (mg of malonic aldehyde kg⁻¹ of fish) were compared with TBA values reported by Campos and Nunes (1993).

RESULTS AND DISCUSSION

The theoretical curve obtained for the ozone concentration in the air inside the desiccators is shown in Fig. 1. The level of ozone during the first 20 min rose rapidly to 0.25 × 10⁻³ g l⁻¹ and then stabilized at 0.27 × 10⁻³ g l⁻¹.

The effects of ozone treatments 1 and 2 on different microflora on fish skin and muscle are shown in Figs 2 and 3, respectively. Treatment 1 did not show a reduction for all

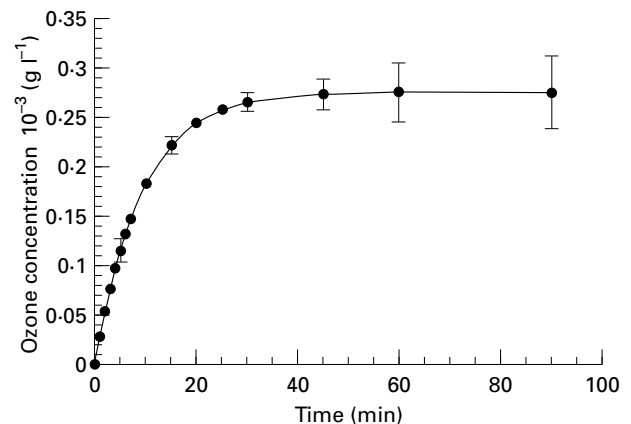


Fig. 1 Ozone concentration in air in desiccators with time obtained by ozone machine

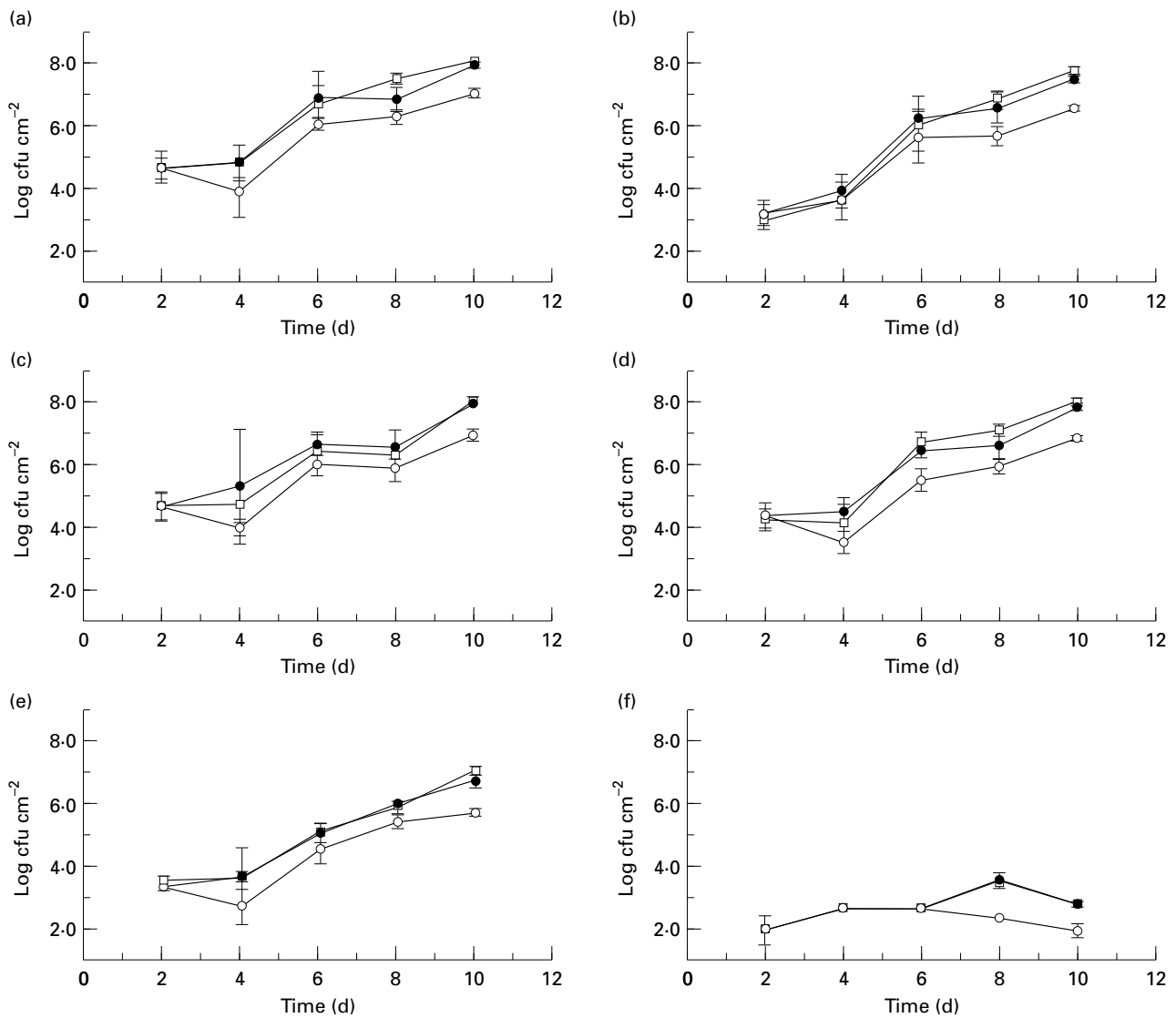


Fig. 2 The effect of different ozone treatments on fish skin microflora. Fish not treated (\ominus), fish treated with one ozone treatment (\bullet), and fish treated with ozone daily (\circ). (a) Total Viable Count. (b) Enterobacteriaceae. (c) Obligate psychrophiles. (d) Pseudomonadaceae. (e) Bacteria H₂S producers. (f) Lactic acid bacteria

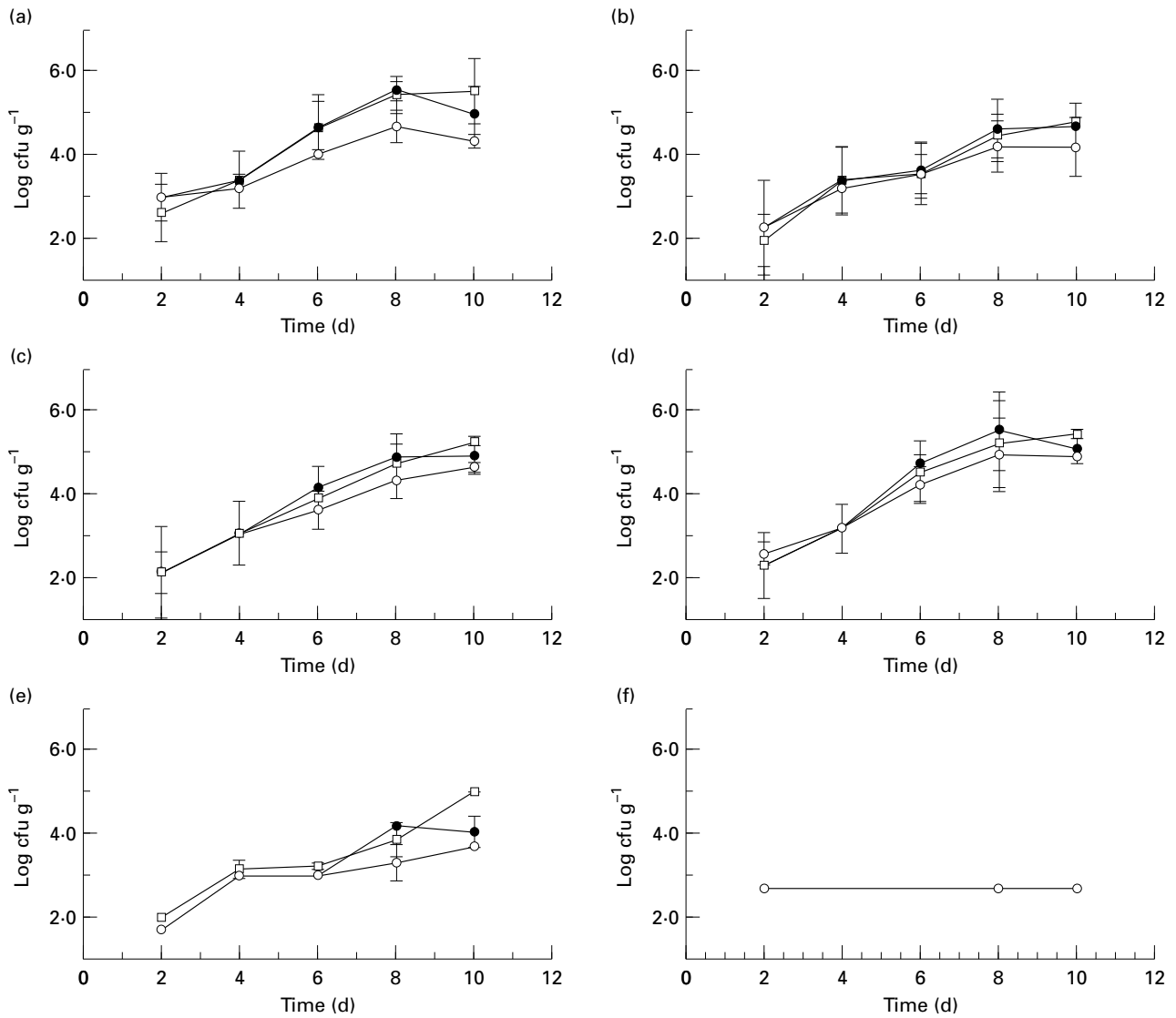


Fig. 3 The effect of different ozone treatments on fish muscle microflora. Fish not treated (⊖); fish treated with one ozone treatment (●); and fish treated with ozone daily (○). (a) Total Viable Count. (b) Enterobacteriaceae. (c) Obligate psychrophiles. (d) Pseudomonadaceae. (e) Bacteria H₂S producers. (f) Lactic acid bacteria

groups of micro-organisms tested, and did not differ statistically ($P > 0.05$) from fish stored on ice (control). Treatment 2 showed a decrease (of up to $1.0 \log \text{cfu cm}^{-2}$ after 10 d) for all groups of micro-organisms examined. These results are significantly different ($P < 0.05$) from controls. Using the same fish species and ozone in 3% NaCl solution, Haraguchi *et al.* (1969) found that, initially, the viable count of bacteria on the surface of the treated fish dropped by 2–3 log cfu compared with controls, extending the storage life of the fish 1.2–1.6 times. The same results were obtained by other investigators working with different seawater species using ozonated ice (Nelson 1982; De Witt *et al.* 1984) or ozone in atmospheres (Rice *et al.* 1982; Mitsuda *et al.* 1991; Dondo *et al.* 1992).

The effect of ozone on fish muscle showed that there was no significant decrease in microbial counts compared with controls, with the exception of Total Viable Counts and H_2S producers which showed a decrease of $1.0 \log \text{cfu g}^{-1}$ and more than $1.0 \log \text{cfu g}^{-1}$, respectively. Results show that ozone treatment had little effect on the bacterial count of whole fish muscle, and are in agreement with previous reports (Haraguchi *et al.* 1969; Ravesi *et al.* 1987).

The effect of fish on board under ozone atmospheres ($0.25 \times 10^{-3} \text{g l}^{-1}$) and treated daily (treatment 3) on different microflora on fish skin and muscle are shown in Fig. 4a, b. Results show an extension in the lag phase during the first 5 days storage for all the micro-organisms; following this period, growth curves were similar to those shown in Figs 2 and 3, but lower microbial numbers were observed.

Results for TBA, pH and sensory analyses for different groups are shown in Table 1. Fish submitted to treatment 2 gave higher sensory scores and differed statistically ($P < 0.05$) from treatment 1 and control fish. Sensory analyses indicated that treatment 3 presented high sensory scores, resulting in an extension of shelf-life of more than 2 days.

Rancidness was never detected by sensory analyses and none of the fish treated developed significant differences in pH and TBA values compared with controls. The TBA values remained low (less than 2.5mg of malonic aldehyde kg^{-1} of fish) throughout the storage period for all different fish treatments, indicating that no accentuated oxidation of fatty acids occurred. Campos and Nunes (1993) reported that TBA values higher than 7.0mg of malonic aldehyde kg^{-1} of fish indicated accentuated oxidation of fatty acids. Ravesi *et al.* (1987) indicated that the very brief contact of the ozone with fish did not result in oxidation of unsaturated lipid material.

The antimicrobial effects of ozone against three Gram-negative bacteria (*Ps. putida*, *S. putrefaciens* and *Enterobacter* sp.), two Gram-positive bacteria (*B. thermosphacta* and *Lact. plantarum*), and a cocktail of these, are shown in Fig. 5a. The data clearly show that destruction of vegetative cells occurs at approximately the same time as exposure to ozone and that

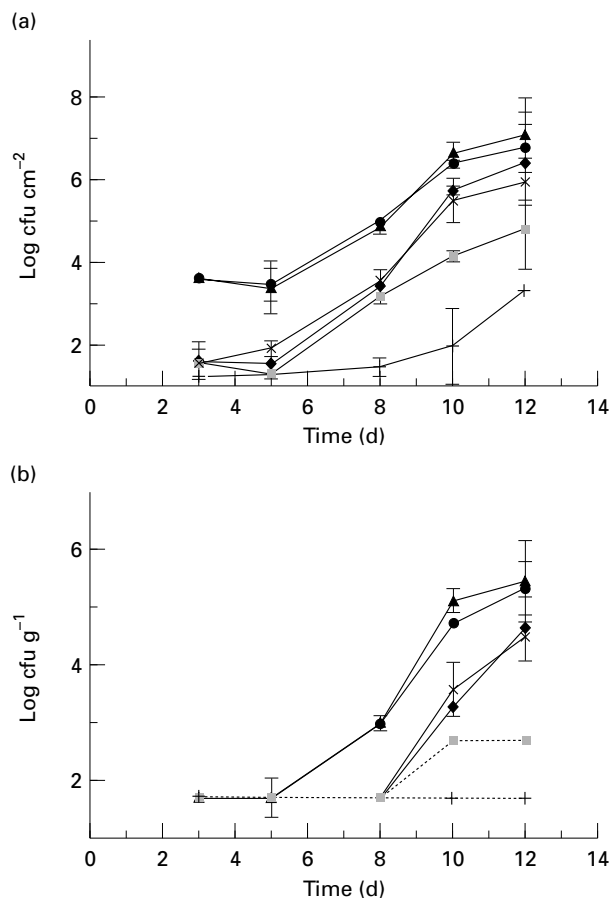


Fig. 4 The effect of ozone atmospheres on board and daily ozone treatment on microflora of (a) fish skin and (b) fish muscle. Total Viable Count (●); obligate psychrophiles (▲); Enterobacteriaceae (■); Pseudomonadaceae (◆); H_2S producers (×); and lactic acid bacteria (l)

destruction increases once a critical concentration is attained. Results showed that the survival rate was not linearly related to ozonation time. The higher death occurred during the first 15 min of ozone exposure. Several other studies using ozone have also shown that death rate kinetics for a variety of bacteria and viruses exhibit a biphasic process over an extended time period (Broadwater *et al.* 1973; Ishizaki *et al.* 1986).

In control experiments where the same experiment was conducted with the same air flow and the ozone lamp switched off, there were no losses of viability during 90 min of exposure (Fig. 5b) in any bacteria except *S. putrefaciens* and *Lact. plantarum*.

The antimicrobial effect of ozone on two stages of bacterial growth are shown in Fig. 6a, b. Exposure of the bacterial cultures to ozone revealed two different patterns initially.

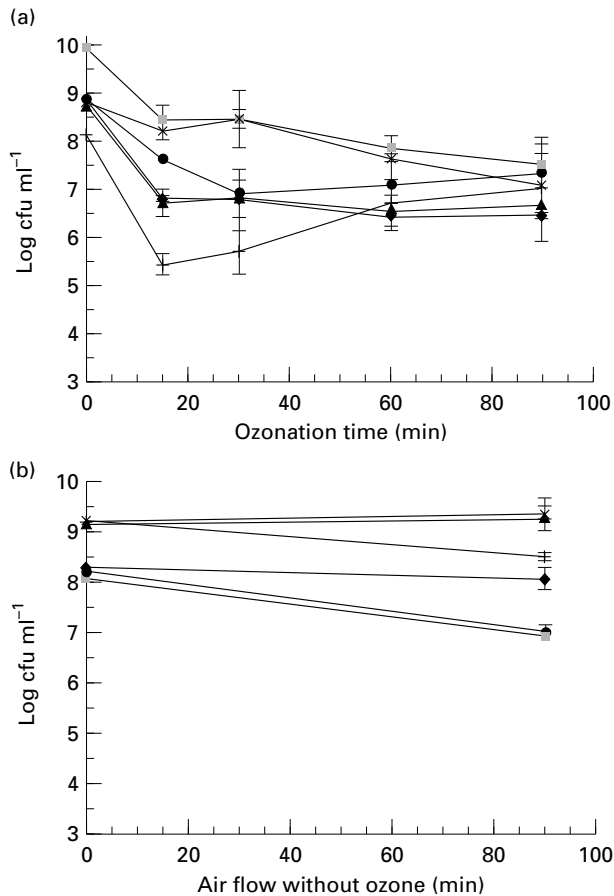


Fig. 5 Survivor curves obtained for each bacterial culture after inoculation onto plates followed by immediate exposure to (a) ozonated air and (b) unozonated air. *Shewanella putrefaciens* (●); *Enterobacter* sp. (◆); *Pseudomonas putida* (■); *Brochothrix thermosphacta* (▲); *Lactobacillus plantarum* (◻); cocktail culture (×)

Enterobacter sp., *Ps. putida* and the cocktail of cultures were not sensitive whereas the other cultures lost viability. Survivor curves for *Lact. plantarum*, *B. thermosphacta* and *S. putrefaciens* showed a similar shape to curves presented in Fig. 5a. *Shewanella putrefaciens* was the most sensitive of the bacteria tested to ozone at this stage of growth. Survivor colonies in the cocktail culture were typical of *Ps. putida*, suggesting that this organism is more ozone-resistant than the others in the cocktail. Following growth on N.a. on plates for 10 h at 20 °C, cells were not sensitive to ozone (Fig. 6b).

Results suggest that sensitivity to ozone is dependent on the stage of cell growth. A different methodology used by Ingram and Barnes (1954) and Foegeding (1986) also found differences in sensitivity of cells at different stages of growth to ozone treatments. It has also been demonstrated that sensitivity of bacteria to various treatments is dependent upon

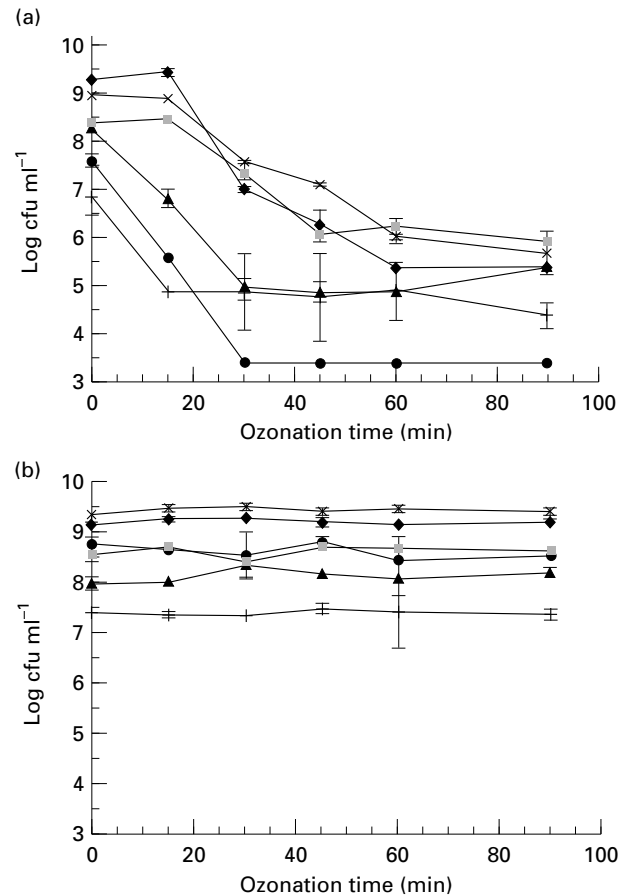


Fig. 6 Survivor curves obtained for each bacterial culture (a) following 4 h of growth on plates and (b) following 10 h of growth on plates, and then exposure to ozonated air. *Shewanella putrefaciens* (●); *Enterobacter* sp. (◆); *Pseudomonas putida* (■); *Brochothrix thermosphacta* (▲); *Lactobacillus plantarum* (◻); cocktail culture (×)

other environmental factors, on the type of organism and the stage of cellular growth (Mackey 1984). Cells at the exponential phase are the most sensitive (Tortora *et al.* 1993).

The bactericidal effect of ozone is shown to be dependent on ozone concentration, the use of different contact periods and the different methodology used. The use of low ozone concentrations in solutions decreases the half-life of ozone and in some cases, the bactericidal effect is not observed. The decomposition of ozone in air is much less than in solution (Hoigné and Bader 1975; Glaze 1986) and may react with other components on the surface of the fish to produce a bactericidal effect. Ravesi *et al.* (1987) reported that ozone may react with some components in sea water to produce a bactericidal ion or compound. Some other authors have reported that ozone treatment stabilized the bacterial surface, reducing these values during several days of refrigerated stor-

Table 1 Sensory scores (\pm SD), TBA (mg of malonic aldehyde per kg of fish) (\pm SD) and pH (\pm SD) values of scad treated with ice (control), one ozone treatment (treatment 1), one ozone treatment and daily ozone exposure (treatment 2) and ozonated on board and ozone treated daily (treatment 3)

No ozonated fish on board				Ozonated fish on board			
Days storage	Sensory scores	TBA	pH	Days storage	Sensory scores	TBA	pH
2				3			
Control	2.70 \pm 0.14	1.05 \pm 0.24	6.11 \pm 0.02	Treatment 3	2.80 \pm 0.24	0.40 \pm 0.02	6.32 \pm 0.15
Treatment 1	2.69 \pm 0.10	0.67 \pm 0.11	6.23 \pm 0.14				
Treatment 2	2.69 \pm 0.10	0.67 \pm 0.11	6.23 \pm 0.14				
4				5			
Control	1.80 \pm 0.08	1.62 \pm 0.26	6.18 \pm 0.10	Treatment 3	2.11 \pm 0.09	0.75 \pm 0.00	6.26 \pm 0.04
Treatment 1	1.64 \pm 0.25	1.13 \pm 0.04	6.25 \pm 0.03				
Treatment 2	1.78 \pm 0.20	1.09 \pm 0.10	6.22 \pm 0.05				
6				8			
Control	1.23 \pm 0.13	1.68 \pm 0.08	6.12 \pm 0.10	Treatment 3	1.68 \pm 0.14	0.73 \pm 0.07	6.14 \pm 0.12
Treatment 1	1.10 \pm 0.14	2.03 \pm 0.14	6.17 \pm 0.08				
Treatment 2	1.23 \pm 0.22	1.30 \pm 0.02	6.15 \pm 0.01				
8				10			
Control	0.99 \pm 0.16	2.49 \pm 0.18	6.31 \pm 0.15	Treatment 3	1.23 \pm 0.22	0.70 \pm 0.06	6.47 \pm 0.10
Treatment 1	0.93 \pm 0.27	1.34 \pm 0.26	6.37 \pm 0.00				
Treatment 2	0.88 \pm 0.29	2.21 \pm 0.25	6.31 \pm 0.06				
10				12			
Control	0.70 \pm 0.07	1.56 \pm 0.04	6.43 \pm 0.02	Treatment 3	0.60 \pm 0.27	1.30 \pm 0.00	6.42 \pm 0.12
Treatment 1	0.75 \pm 0.12	1.42 \pm 0.14	6.45 \pm 0.01				
Treatment 2	0.81 \pm 0.03	1.95 \pm 0.04	6.58 \pm 0.10				

age, thus improving quality (Sheldon and Brown 1986; Mitsuda *et al.* 1991; Beuchat 1992; Dondo *et al.* 1992). In the present study, the gaseous ozone had a bactericidal effect on five fish bacteria and surface microflora at the ozone concentration tested.

The use of a gaseous ozone system under practical conditions appears to be a viable option for fisherman to improve catch quality and marketability.

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REFERENCES

American Public Health Association (1975) Ozone (residual). In *Standard Methods For the Examination of Water and Wastewater*,

13th edn. pp. 426–428. Washington DC: American Public Health Association Inc.

Anon. (1989) Commission Regulation (EEC) no. 3506/89. *Official Journal of the European Communities* 32, 11–12.

Anon. (1990) Fish and fish products. Determination of TBA value, spectrophotometric method. *Instituto Portuguese of Quality* NP 3356.

Beuchat, L.R. (1992) Surface disinfection of raw produce. *Dairy Food and Environmental Sanitation* 12, 6–9.

Broadwater, W.T., Hoehn, R.C. and King, P.H. (1973) Sensitivity of three selected bacterial species to ozone. *Applied Microbiology* 26, 391–393.

Campos, R.M. and Nunes, M.L. (1993) Evolução de alguns índices de frescura em várias espécies pelágicas durante a armazenagem em refrigerado. Comunicação poster. Encontro de Química dos Alimentos, Santarém.

Chen, H.C., Huang, S.H., Moody, M.W. and Jiang, S.T. (1992) Bactericidal and mutagenic effects of ozone on shrimp (*Penaeus monodon*) meat. *Journal of Food Science* 57, 923–927.

Dalgaard, P. (1995) Qualitative and quantitative characterization of spoilage bacteria from packed fish. *International Journal of Food Microbiology* 26, 319–333.

Dewitt, B.J., McCord, V., Holt, B.L. Ellis, D.K., Finne, G. and

- Nickelson, R. (1984) The potential use of ozonated ice for on board storage of Gulf of Mexico shrimp. In *Nineteenth Annual Tropical Subtropical Fish Technology Conference, Texas, USA*, University Sea Grant Republic.
- Dondo, A., Nachtman, C., Doglione, L., Rosso, A. and Genetti, A. (1992) Foods: their preservation by combined use of refrigeration and ozone. *Ingegneria alimentare le Conserve Animali* **8**, 16–25.
- Foegeding, P.M. (1985) Ozone inactivation of *Bacillus* and *Clostridium* spore populations and the importance of the spore coat to resistance. *Food Microbiology* **2**, 123–134.
- Glaze, W.H. (1986) Reaction products of ozone: a review. *Environmental Health Perspectives* **69**, 151–157.
- Gram, L., Trolle, G. and Huss, H.H. (1987) Detection of specific spoilage bacteria from fish stored at low (0 °C) and high (20 °C) temperatures. *International Journal of Food Microbiology* **4**, 65–72.
- Greene, A.K., Few, B.K. and Serafini, J.C. (1993) A comparison of ozonation and chlorination for the disinfection of stainless steel surfaces. *Journal of Dairy Science* **76**, 3617–3620.
- Greer, G.G. and Jones, S.D.M. (1989) Effects of ozone on beef carcass shrinkage, muscle quality and bacterial spoilage. *Canadian Institute of Food Science and Technology Journal* **22**, 156–160.
- Haraguchi, T., Simidu, V. and Aiso, K. (1969) Preserving effect of ozone to fish. *Bulletin of the Japanese Society of Scientific Fisheries* **35**, 915–919.
- Hobbs, G. (1991) Fish: microbiological spoilage and safety. *Food Science and Technology Today* **5**, 166–173.
- Hoigné, J. and Bader, H. (1975) Ozonation of water: role of hydroxyl radicals as oxidizing intermediates. *Science* **190**, 782–784.
- Ingram, M. and Barnes, E.M. (1954) Sterilization by means of ozone. *Journal of Applied Bacteriology* **17**, 246–271.
- Ishizaki, K., Shinriki, N. and Matsuyama, H. (1986) Inactivation of *Bacillus* spores by gaseous ozone. *Journal of Applied Bacteriology* **60**, 67–72.
- Mackey, B.M. (1984) Lethal and sublethal effects of refrigeration, freezing and freeze-drying on micro-organisms. In *The Revival of Injured Microbes* ed. Andrew, M.H.E. and Russell, A.D. pp. 45–73. London: Academic Press, Inc.
- Miles, A.A. and Misra, S.S. (1938) The estimation of the bactericidal power of the blood. *Journal of Hygiene* **38**, 732–749.
- Mitsuda, H., Ohminami, H. and Nagasawa, H. (1991) Process for sterilizing food stuffs. United States Patent no. 5.011.699. Japan Food Industry Association Inc.
- Nelson, W. (1982) The use of ozonated ice to extend the shelf life of fresh Alaskan fish. *Republic Submission Alaska Department Commercial Economic Development, Officially Commercial Fish Development Anchorage*.
- Ravesi, E.M., Licciardello, J. J. and Racicot, L.D. (1987) Ozone treatments of fresh Atlantic cod, *Gadus morhua*. *Marine Fisheries Review* **49**, 37–42.
- Rice, R.G., Farquhar, J.W. and Bollyky, L.J. (1982) Review of the applications of ozone for increasing storage times of perishable foods. *Ozone Science and Engineering* **4**, 147–163.
- Sheldon, B.W. and Brown, A.L. (1986) Efficacy of ozone as a disinfectant for poultry carcasses and chill water. *Journal of Food Science* **51**, 305–312.
- Tassou, C.C., Drosinos, E.H. and Nychas, G.J.E. (1995) Inhibition of resident microbial flora and pathogen inoculate on cold fresh fish in olive oil, oregano, and lemon juice under modified atmosphere or air. *Journal of Food Protection* **59**, 31–34.
- Tortora, G.J., Funke, B.R. and Case, C.L. (1993) Crecimiento bacteriano. In *Introduccion a la Microbiologia* ed. pp. 144–167. Zaragoza: The Benjamin Cummings Publishing Company.