

# Ozone Treatments of Fresh Atlantic Cod, *Gadus morhua*

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## Introduction

The strong oxidizing nature of ozone, known since its discovery in 1840, has made it a useful agent for seawater disinfection (Blogoslawski et al., 1975; Blogoslawski, 1977) by effectively destroying bacteria, yeasts, and viruses. Its potential value for shellfish depuration was first reported by Voille (1929) and later by Salmon and Le Gall (1936). More recently, Blogoslawski and Stewart (1977) cited in a review article numerous reports of the successful use of ozone to 1) control microbial populations in closed marine systems, 2) depurate shellfish, 3) improve water quality and prevent disease in aquaculture, 4) inactivate red tide toxins, and 5) prevent biofouling, i.e. the rapid and undesirable growth of aquatic flora and fauna which can plug mechanical systems.

Ozone has been reported in numerous studies to extend the storage life of many perishable foods by slowing decomposition caused by microorganisms. These studies were reviewed by Farquhar and Rice (1982). Storage life extension of fish using ozone was first reported by the French investigators Salmon and Le Gall (1936). They found they could extend the storage life of whiting 4 days by rinsing

the fish with freshly ozonated seawater and then packing them in small boxes with ozonated ice.

Interest in the use of ozone for fish quality preservation was not then sustained, but resumed about 30 years later when Japanese investigators (Haraguchi et al., 1969) reported a 4-day storage life extension by ozonation of brine-dipped jack mackerel and striped mackerel.

In a more recent study (Nelson, 1982), tests were conducted to determine whether the storage life of freshly caught Alaska salmon, *Oncorhynchus* spp., could be extended by packing the fish in ice made from ozonated water compared with fish conventionally packed in ice made from nonozonated water. The bacterial count on the fish kept in ozonated ice ( $94 \times 10^3/g$ ) was only 3 percent of that on fish packed in regular ice ( $2.7 \times 10^6/g$ ) after 8-9 days storage at 28°F. The results of this study indicated that the fresh quality of ozone-iced Pacific salmon can be maintained up to 6 days. And, DeWitt et al. (1984) report a "possible" extension of 1-2 days in the storage life of Gulf of Mexico shrimp with the use of ozonated ice.

The nature and rate of deterioration varies considerably with different species (Bramsnaes, 1965); therefore, the purpose of our study was to determine if the use of ozone could, by various treatments, extend the storage life of gutted cod as it appears to do with some other species of fish.

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## Materials and Methods

This study was conducted in two parts. For Part I, 36 eviscerated cod (3.5-6 pounds each) 1-day post mortem, were procured from a Gloucester, Mass., day boat in March, 1984. These fish were held in ice for 24 hours while fresh ozonated ice was prepared. They were then sprayed with tap water and divided into three treatment groups of 12 fish each. The treatments were: 1) Control samples stored in freshwater flake ice, 2) rinsed for one minute with 500 ml of freshly prepared ozonated water, while suspended by their tails, then stored in flake ice, 3) stored in 2 inch cube-sized chunks of ozonated ice.

The fish were stored in commercial plastic fish boxes (125-pound capacity). Each box contained six fish divided into two layers of three fish and was covered with a sheet of polyethylene during storage. The boxes were kept at an ambient temperature of about 37°F. Two fish from each group were tested after 0, 5, 9, 13, 16, and 20 days of treatment (2, 7, 11, 15, 18, and 22 days post mortem). One fillet from each fish was used for bacteriological analyses and sensory evaluation, and the second was used for pH and chemical tests. All tests were conducted in duplicate.

For Part II of the study, 26 market-sized eviscerated cod 1 day post mortem were purchased from a local day-boat in June 1984. Two fish were reserved for examination and testing as 0 day reference samples. The remaining fish, after rinsing with tap water, were divided into four groups of six, weighed and put into each of four plastic barrels (32-gallon capacity) containing chilled seawater

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**ABSTRACT**—*The effect of ozone was investigated by various applications on the iced storage life of fresh gutted Atlantic cod, Gadus morhua. The different treatments incorporated the ozone in either the ice, rinse water, or chilled seawater. Shelf life, as assessed by sensory, chemical, and microbiological tests, was not sufficiently extended by any of the treatments.*

(CSW). The fish:seawater:ice ratio in these containers was 3:1.5:1. These barrels were evenly divided into two treatment groups: Group 1 (control) was aerated with purified compressed air, and group 2 was ozonated with gaseous ozone. The barrels were stored at 37°F and daily, for 7 days, the appropriate gas was bubbled for 20 minutes into these barrels through a 12-inch long air stone placed at the bottom of each. Two fish from each group were tested after 0, 1, 4, and 7 days of treatment. After 7 days, the remaining fish were removed from the chilled seawater, stored in flake ice in covered commercial plastic fish boxes, and testing continued during an additional 8 days storage.

The ozone used in this study was produced in a Welsbach Ozonator<sup>1</sup> (Model 408) using purified oxygen as the feed gas. The ozonated water (about 8.0 ppm) was produced by bubbling the gaseous ozone through an air stone into a stream of water flowing through an 18-foot long 1½-inch PVC pipe. To enhance saturation of the water with gas, the pipe was packed with small stones and it contained four U-shaped bends with ¼-inch restrictors. It was collected in a covered nalgene carboy containing flake ice and was drawn from a spigot at the bottom of the carboy. The ozonated chunk ice (about 0.6 ppm O<sub>3</sub>) was made by chopping blocks of ice prepared by filling polyethylene-lined metal trays (12×42×2 inches) with ozonated water and freezing these rapidly in a plate freezer. The procedure for testing the fish in Part II of the study was similar to that followed in Part I.

For sensory analyses, each fish, and subsequently its skinned fillets, was evaluated in the raw state. Examination was made of the following characteristics: 1) General external appearance of the fish, 2) condition of the eyes, 3) appearance and odor of the gills, 4) condition of the muscle, resistance to pressure, gaping, and adherence to the skeletal bone, 5) appearance and odor of the abdominal cavity, 6) color of the muscle—discoloration and blood spots, and 7)

<sup>1</sup>Mention of trade names on commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

odor of the muscle.

The fillets were then steamed in foil-covered pans at 212°F for 15 minutes and evaluated by a 6-member panel of laboratory staff members for appearance, odor, flavor, and texture. Samples were rated on a scale from 1 (inedible) to 9 (excellent). When the average score of any of the above attributes decreased to a value of 5.5 (between fair and borderline) the end of the storage life was considered reached.

To obtain pH, a 20 g sample of fish muscle was blended with 40 ml of distilled water for 1 minute and the pH of the homogenate was measured with a Fisher Model 320 expanded scale pH meter.

Dimethylamine (DMA) and trimethylamine (TMA) analyses were performed by gas chromatography using N-propylamine as the internal standard (Lundstrom and Racicot, 1983). Results are expressed as MgN/100 g muscle.

Thiobarbituric acid (TBA) reactive substances were determined by the method of Yu and Sinnhuber (1957), modified by the addition of disodium ethylenediamine tetracetate (EDTA) and propyl gallate to prevent oxidation during blending. TBA number was calculated by the procedure reported by Sinnhuber and Yu (1958).

The concentration of ozone (O<sub>3</sub>) in water and ice was determined by an iodometric method (APHA, 1971). Measured volumes of water and weighed amounts of ice were transferred to 8-ounce jars containing the necessary volume of potassium iodide solution, and the jars were immediately capped. When ice was tested, the jar were set in pans of tepid water to hasten melting. The ozone concentration was measured immediately after melting was complete. Ozonated chunk ice was used in this study because we were unable to measure residual ozone in flake ice which had been prepared from water with an ozone concentration as high as 8.0 ppm. The presence of ozone in the chunk ice was short lived and was not measurable after 24 hours. About 50 percent of the originally measured ozone disappeared after one hour.

The aerobic plate count (APC) was made from appropriate dilutions onto pour plates of TPE agar (Standard Methods Agar reinforced with 0.5 percent

Bacto-peptone and 0.5 percent NaCl) as recommended by Lee and Pfeifer (1974) for seafoods. Duplicate plates were incubated at 68°F and colony counts were made after 5 days.

## Results and Discussion

The use of ozone in rinse water and/or in ice are procedures which could be used by fishermen aboard vessels or by processors in their plants. The application of ozone to chilled seawater might enable fishermen to sustain high quality in their catches when trips of several days are necessary. It is understandable that if ozonation is to benefit the storage life of fish, the sooner it is applied, the greater will be its advantage. However, its possible benefit to fish of several days post mortem age cannot be discounted as Castell (1953) showed that percentage bacterial reduction on fillets as a result of washing round fish by various means was low for recently caught iced fish and relatively high for fish which had been in ice for several days. Circumstances prevented us from studying fish immediately after their removal from water. Prior to treatment and storage, all fish were examined and judged by raw sensory evaluation to be in excellent condition. For the duration of Part I of the study, no perceptible sensory difference among any of the treatments was noted in the raw whole fish or fillets.

The sensory panel scores (Table 1) of cooked cod treated with ozone in various ways and stored in ice for Part I of this study are shown. There was no significant difference in any of the attributes due to treatments at any time other than at the 9th day if treatment when the controls were significantly lower (1 percent level) in flavor score from the other two treatments. This difference was not maintained so that neither the ozone-rinse nor the ozone-ice appears to extend the panels acceptance of the iced cod. The results of chemical, pH, and bacteriological analyses for Part I of this study are summarized in Table 2.

There was a large deviation in values obtained from fillets of the same treatment for all three amines throughout the study. All treatments showed an increase in TMA content (Fig. 1) after 5 days. After 13 days, the ozone-rinsed fish had a slightly higher content of TMA than the

control, while a little less TMA was found in the ozone-iced fish, but differences were not significant.

The DMA content of the cod fillets was low throughout the study (less than 3.9 mgN/100 g for all treatments, but as can be seen from Figure 2, the treatments had no effect on DMA content. Salmon and Le Gall (1936), using either cod or whiting (not stated), did find a lower concentration of TMA and total volatile nitrogen throughout a 12-day storage period in fish stored in ozonated ice compared with ordinary ice. It must be

noted, however, that the "ordinary" ice which they used was known to be far from sterile, having a presence of about 25,000 organisms/cc, among which *Pseudomonas fluorescens* were dominant. Fish stored on this nonsterile ice was judged inedible at the 12th day of storage, while those on the sterilized ice were not considered objectionable until the 16th day.

Rancidity did not develop in any of the fish regardless of treatment. All showed a very gradual and very small increase in TBA number throughout the 20 day stor-

age period. Nelson (1982) reported a lower malonaldehyde concentration in fresh Alaskan salmon (pink, coho, sockeye, and silver) stored in ozonated ice compared with regular ice. However, unlike with salmon, rancidity is not a problem with conventionally iced cod and our concern was in whether ozone would cause it to develop. Our results indicate that the very brief contact of the ozone with the fish did not result in oxidation of unsaturated lipid material. It is highly unlikely that the ozone ever contacted fatty acids.

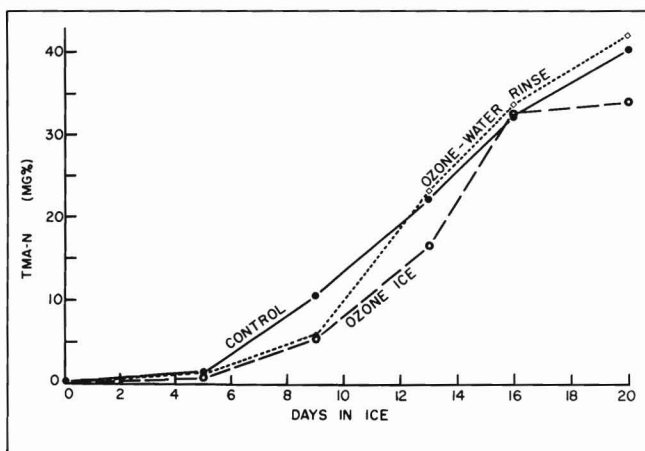


Figure 1.—Trimethylamine content of gutted cod treated with ozone in various manners and stored in ice.

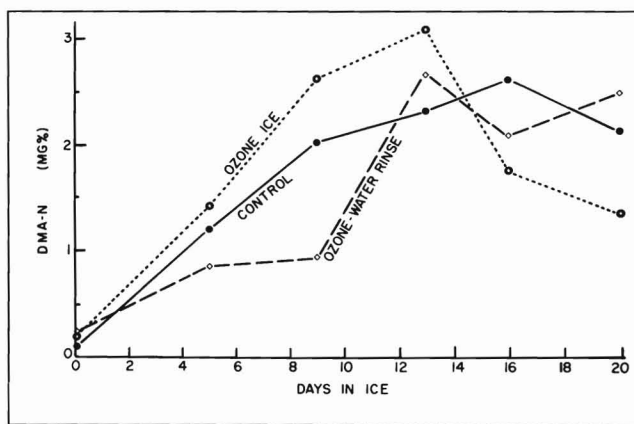


Figure 2.—Dimethylamine content of gutted cod treated with ozone in various manners and stored in ice.

Table 1.—Sensory scores ( $\pm$ SD) for appearance, odor, flavor, and texture of cooked cod treated with ordinary ice (Control), or ozonated water (Rinsed), or ozonated ice (Iced).

Days storage	Treatment	Appearance	Odor	Flavor	Texture
0	Control	8.4 $\pm$ 0.8	8.3 $\pm$ 0.8	8.5 $\pm$ 0.8	7.8 $\pm$ 1.3
	Rinsed	8.4 $\pm$ 0.8	8.5 $\pm$ 0.8	8.5 $\pm$ 0.8	8.0 $\pm$ 1.1
5	Control	7.7 $\pm$ 0.8	7.8 $\pm$ 0.8	7.6 $\pm$ 0.9	7.7 $\pm$ 0.9
	Rinsed	7.8 $\pm$ 0.7	7.6 $\pm$ 1.0	7.8 $\pm$ 0.6	7.7 $\pm$ 0.9
	Iced	8.0 $\pm$ 0.9	7.8 $\pm$ 0.9	7.8 $\pm$ 0.7	7.7 $\pm$ 0.9
9	Control	6.3 $\pm$ 1.1	6.3 $\pm$ 1.2	6.3 $\pm$ 0.9	6.2 $\pm$ 0.9
	Rinsed	7.4 $\pm$ 0.5	7.3 $\pm$ 0.5	7.1 $\pm$ 0.6	6.8 $\pm$ 0.9
	Iced	7.1 $\pm$ 0.7	7.0 $\pm$ 0.7	7.2 $\pm$ 0.8	7.2 $\pm$ 0.7
13	Control	5.7 $\pm$ 1.4	5.4 $\pm$ 1.4	6.3 $\pm$ 1.6	5.8 $\pm$ 1.4
	Rinsed	5.9 $\pm$ 1.7	5.5 $\pm$ 1.5	5.83 $\pm$ 1.0	6.0 $\pm$ 1.0
	Iced	5.9 $\pm$ 1.8	5.7 $\pm$ 1.2	5.4 $\pm$ 1.4	5.6 $\pm$ 1.2
16	Control	5.4 $\pm$ 1.0	4.3 $\pm$ 2.2	4.1 $\pm$ 2.0	4.5 $\pm$ 2.4
	Rinsed	5.9 $\pm$ 0.7	4.2 $\pm$ 1.9	4.4 $\pm$ 2.1	4.6 $\pm$ 2.2
	Iced	6.3 $\pm$ 1.0	4.2 $\pm$ 2.2	4.3 $\pm$ 2.1	4.6 $\pm$ 2.2
20	Control	5.1 $\pm$ 1.4	2.6 $\pm$ 1.6	3.5 $\pm$ 2.3	3.9 $\pm$ 2.2
	Rinsed	4.3 $\pm$ 2.2	1.8 $\pm$ 0.9	1.3 $\pm$ 1.5	3.1 $\pm$ 2.0
	Iced	4.0 $\pm$ 2.2	1.5 $\pm$ 0.8	1.7 $\pm$ 1.0	2.7 $\pm$ 2.2

Table 2.—Chemical and bacteriological analyses and pH values of cod treated with ordinary ice (Control), an ozonated rinse, (Rinsed), and ozonated ice (Iced).

Days storage	Treatment (mgN/100g)	TMA (mgN/100g)	DMA (mgN/100g)	TMAO (mgN/100g)	TBA no. (20°C)	APC (20°C)	pH
0	Control	0.18 $\pm$ 0.08	0.10 $\pm$ 0.06	83.6 $\pm$ 0.4	0.50 $\pm$ 0.13	9.4( $\pm$ 3) $\times$ 10 <sup>3</sup>	6.6 $\pm$ 0.4
	Rinsed	0.12 $\pm$ 0.02	0.25 $\pm$ 0.12	85.7 $\pm$ 2.4	0.60 $\pm$ 0.06	7.7( $\pm$ 8.1) $\times$ 10 <sup>3</sup>	5.8 $\pm$ 0.0
5	Control	1.19 $\pm$ 0.53	1.20 $\pm$ 0.29	76.2 $\pm$ 1.1	0.54 $\pm$ 0.0	187.3( $\pm$ 134.0) $\times$ 10 <sup>3</sup>	6.7 $\pm$ 0.0
	Rinsed	1.03 $\pm$ 0.73	0.86 $\pm$ 0.17	75.2 $\pm$ 2.4	0.61 $\pm$ 0.09	72.3( $\pm$ 17.1) $\times$ 10 <sup>3</sup>	6.8 $\pm$ 0.1
	Iced	0.67 $\pm$ 0.50	1.42 $\pm$ 0.95	78.8 $\pm$ 6.0	0.62 $\pm$ 0.05	142.1( $\pm$ 161.1) $\times$ 10 <sup>3</sup>	6.6 $\pm$ 0.1
9	Control	10.36 $\pm$ 6.68	2.01 $\pm$ 1.28	59.7 $\pm$ 4.7	0.67 $\pm$ 0.01	14.7( $\pm$ 1.6) $\times$ 10 <sup>6</sup>	7.0 $\pm$ 0.0
	Rinsed	5.52 $\pm$ 5.13	0.93 $\pm$ 0.28	71.8 $\pm$ 13.4	0.69 $\pm$ 0.08	9.4( $\pm$ 5.9) $\times$ 10 <sup>6</sup>	6.8 $\pm$ 0.0
	Iced	5.45 $\pm$ 0.77	2.59 $\pm$ 1.80	66.9 $\pm$ 16.2	0.73 $\pm$ 0.0	1.4( $\pm$ 0.2) $\times$ 10 <sup>6</sup>	6.8 $\pm$ 0.0
13	Control	22.8 $\pm$ 10.70	2.29 $\pm$ 0.44	39.9 $\pm$ 28.2	0.59 $\pm$ 0.06	94.5( $\pm$ 7.8) $\times$ 10 <sup>6</sup>	7.0 $\pm$ 0.1
	Rinsed	22.88 $\pm$ 0.27	2.64 $\pm$ 0.35	35.8 $\pm$ 11.4	0.54 $\pm$ 0.03	37.5( $\pm$ 5.0) $\times$ 10 <sup>6</sup>	7.0 $\pm$ 0.2
	Iced	16.28 $\pm$ 10.73	3.04 $\pm$ 0.13	51.7 $\pm$ 3.0	0.60 $\pm$ 0.01	43.5( $\pm$ 0.7) $\times$ 10 <sup>6</sup>	7.0 $\pm$ 0.1
16	Control	31.79 $\pm$ 10.91	2.58 $\pm$ 0.69	36.1 $\pm$ 5.7	0.65 $\pm$ 0.08	146.5( $\pm$ 132.2) $\times$ 10 <sup>6</sup>	7.1 $\pm$ 0.3
	Rinsed	33.07 $\pm$ 5.9	2.05 $\pm$ 0.57	38.0 $\pm$ 15.3	0.76 $\pm$ 0.06	80.5( $\pm$ 13.4) $\times$ 10 <sup>6</sup>	6.8 $\pm$ 0.1
	Iced	32.13 $\pm$ 0.8	1.72 $\pm$ 0.08	43.9 $\pm$ 3.4	0.72 $\pm$ 0.05	179.5( $\pm$ 88.4) $\times$ 10 <sup>6</sup>	7.0 $\pm$ 0.0
20	Control	39.40 $\pm$ 11.94	2.07 $\pm$ 0.13	19.7 $\pm$ 20.9	0.97 $\pm$ 0.04	556( $\pm$ 601) $\times$ 10 <sup>6</sup>	7.2 $\pm$ 0.2
	Rinsed	41.27 $\pm$ 1.58	2.44 $\pm$ 1.32	13.9 $\pm$ 8.5	0.81 $\pm$ 0.08	106( $\pm$ 36.8) $\times$ 10 <sup>6</sup>	7.3 $\pm$ 0.2
	Iced	33.33 $\pm$ 22.37	1.33 $\pm$ 0.74	33.2 $\pm$ 21.0	0.84 $\pm$ 0.05	332( $\pm$ 166) $\times$ 10 <sup>6</sup>	7.4 $\pm$ 0.3

A gradual increase in pH resulted in the iced controls and both of the ozone treated fish. The rate of increase did not vary with the treatment, just as would be expected from the amine production.

Figure 3 shows the results of aerobic plate count. None of the treatments appear to delay an increase in bacterial counts. Salmon and Le Gall (1936) reported a marked decrease in the number of microorganisms observed on the flesh of whiting immediately following an ozonated seawater wash. This difference was not apparent between the ozone-rinsed and control fish in our study; how-

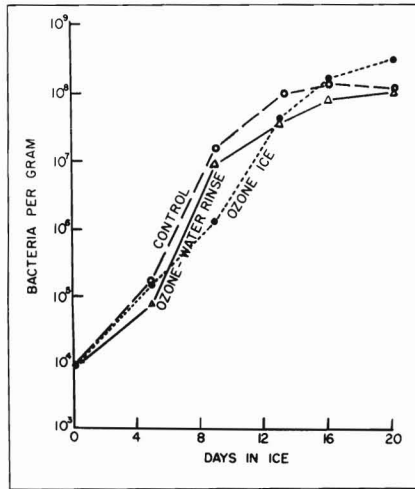


Figure 3.—Aerobic plate count of gutted cod treated with ozone in various manners and stored in ice.

ever, the initial aerobic plate counts in our study were low for all of the fish. Unfortunately, Salmon and Le Gall (1936) did not report bacterial counts after storage. In the study with Alaska fresh salmon, Nelson (1982) reported that when the ozone contacted the skin of the fish, the bacterial counts remained in the tens and hundreds of thousands after 8-9 days of storage on ozonated ice while the controls showed counts in the million-plus range. De Witt et al. (1984), in two separate studies with Gulf of Mexico shrimp, reported that storage in ozonated ice had no effect on the bacterial spoilage of shrimp in one study, but could possibly have a 1-2 day storage life extension effect on their bacteriological spoilage in the second study.

Sensory evaluation scores for cod held for 7 days in either aerated (controls) or ozonated chilled seawater prior to storage on ordinary ice for Part II of this study are summarized in Table 3. Prior to day 7, there was no significant difference in any of the attributes between the different treatments. At days 7 and 11, the flavor and odor of the ozonated fish were rated significantly lower than the flavor and odor of the control samples. Beyond the 11th day of treatment, there was no significant difference between treatments.

Examination of the raw whole fish and fillets did not reveal any differences between the treatments for off-odor development. At day 4, both of the ozonated samples felt less firm and resilient than

the control fish; however, this observation did not seem to influence taste panel results. This softness was still noted in the ozonated samples at 7 and 11 days, but it is not clear from taste test results if this influenced panelists' texture scores. At 7 and 11 days, the skin of ozonated cod seemed more faded or bleached than the controls, but observations on the initial color of individual fish were not made and recorded, so this difference may not be actual. Beyond 11 days, there was no obvious differences in the general condition of the whole fish or fillets.

Table 4 summarizes the results of chemical, pH, and bacteriological analyses for Part II of this study. All of these data, with the exception of pH, would seem to indicate that not only did ozonation, as performed by us, have no storage life extension effect on cod, but rather it may have accelerated deteriorative processes.

Figures 4 and 5 show amine formations. As with the treatments in Part I of this study, the rate of TMA formation was more rapid in the ozonated than in the control fish. It is difficult to explain why ozonated samples had a higher TMA content than controls, since reducing agents usually break down TMAO to TMA. It may be possible that the ozonation treatment was selecting out bacterial species that were capable of producing TMA from TMAO. Unfortunately, there was no sampling done between day 1 and day 4, so it is not known

Table 3.—Sensory scores ( $\pm$ SD) for appearance, odor, flavor, and texture of cooked cod treated prior with aerated CSW plus ice (Control) and ozonated CSW plus ice (Ozonated).

Days storage	Treatment	Appearance	Odor	Flavor	Texture
0	Control	8.7 $\pm$ 0.7	8.6 $\pm$ 0.7	8.1 $\pm$ 0.8	8.1 $\pm$ 1.1
1	Control	8.8 $\pm$ 0.5	8.8 $\pm$ 0.4	8.8 $\pm$ 0.4	7.8 $\pm$ 1.4
	Ozonated	8.8 $\pm$ 0.5	8.7 $\pm$ 0.5	8.7 $\pm$ 0.5	8.3 $\pm$ 0.8
4	Control	7.8 $\pm$ 0.5	7.7 $\pm$ 0.7	7.5 $\pm$ 0.7	7.3 $\pm$ 1.0
	Ozonated	7.4 $\pm$ 0.8	7.7 $\pm$ 0.7	7.4 $\pm$ 0.7	6.9 $\pm$ 1.2
7	Control	7.8 $\pm$ 0.5	7.4 $\pm$ 0.7	7.3 $\pm$ 0.7	7.3 $\pm$ 0.7
	Ozonated	6.6 $\pm$ 0.7	6.2 $\pm$ 1.0	6.2 $\pm$ 1.2	6.4 $\pm$ 0.5
11	Control	6.0 $\pm$ 1.6	6.1 $\pm$ 1.2	6.3 $\pm$ 1.2	5.3 $\pm$ 1.7
	Ozonated	6.3 $\pm$ 0.9	4.7 $\pm$ 1.2	4.9 $\pm$ 1.0	5.8 $\pm$ 0.9
13	Control	6.1 $\pm$ 0.7	5.4 $\pm$ 1.0	5.3 $\pm$ 1.0	4.8 $\pm$ 1.6
	Ozonated	6.3 $\pm$ 0.6	4.2 $\pm$ 1.5	4.4 $\pm$ 1.6	5.5 $\pm$ 1.7
15	Control	5.6 $\pm$ 1.3	3.7 $\pm$ 2.2	3.8 $\pm$ 2.5	4.0 $\pm$ 2.3
	Ozonated	5.8 $\pm$ 0.9	4.4 $\pm$ 1.4	4.7 $\pm$ 1.3	5.3 $\pm$ 1.2

Table 4.—Chemical and bacteriological analyses and pH values ( $\pm$ SD) of cod treated with aerated CSW plus ice (Control) and ozonated CSW plus ice (Ozonated).

Days storage	Treatment (mgN/100g)	TMA (mgN/100g)	DMA (mgN/100g)	TMAO (mgN/100g)	TBA no. (20°C)	APC (20°C)	pH
0	Control	0.35 $\pm$ 0.33	0.09 $\pm$ 0.06	71.8 $\pm$ 7.2	0.65 $\pm$ 0.05	7.3( $\pm$ 6.0) $\times$ 10 <sup>3</sup>	6.7 $\pm$ 0.3
1	Control	0.12 $\pm$ 0.06	0.41 $\pm$ 0.52	70.5 $\pm$ 21.1	0.71 $\pm$ 0.06	9.2( $\pm$ 3.7) $\times$ 10 <sup>3</sup>	6.8 $\pm$ 0.3
	Ozonated	0.30 $\pm$ 0.14	0.18 $\pm$ 0.06	64.8 $\pm$ 1.6	0.88 $\pm$ 0.01	1.7( $\pm$ 0.4) $\times$ 10 <sup>3</sup>	6.7 $\pm$ 0.2
4	Control	0.29 $\pm$ 0.11	0.46 $\pm$ 0.15	70.2 $\pm$ 4.2	0.72 $\pm$ 0.05	6.5( $\pm$ 1.6) $\times$ 10 <sup>3</sup>	7.0 $\pm$ 0.4
	Ozonated	0.25 $\pm$ 0.10	0.56 $\pm$ 0.41	66.4 $\pm$ 4.2	0.60 $\pm$ 0.08	16.0( $\pm$ 4.2) $\times$ 10 <sup>3</sup>	6.8 $\pm$ 0.2
7	Control	2.88 $\pm$ 2.23	0.77 $\pm$ 0.19	51.3 $\pm$ 16.7	0.69 $\pm$ 0.06	118.5( $\pm$ 140.7) $\times$ 10 <sup>3</sup>	6.6 $\pm$ 0.0
	Ozonated	15.52 $\pm$ 2.76	1.10 $\pm$ 0.33	38.2 $\pm$ 4.2	0.74 $\pm$ 0.01	606.0( $\pm$ 199.4) $\times$ 10 <sup>3</sup>	7.0 $\pm$ 0.3
11	Control	16.20 $\pm$ 6.47	1.11 $\pm$ 0.54	32.8 $\pm$ 7.4	0.85 $\pm$ 0.06	2.0( $\pm$ 1.7) $\times$ 10 <sup>6</sup>	6.9 $\pm$ 0.4
	Ozonated	35.03 $\pm$ 5.71	1.40 $\pm$ 0.12	4.2 $\pm$ 4.1	1.05 $\pm$ 0.05	5.3( $\pm$ 3.1) $\times$ 10 <sup>6</sup>	6.7 $\pm$ 0.2
11	Control	16.20 $\pm$ 6.47	1.11 $\pm$ 0.54	32.8 $\pm$ 7.4	0.85 $\pm$ 0.06	2.0( $\pm$ 1.7) $\times$ 10 <sup>6</sup>	6.94 $\pm$ 0.5
	Ozonated	35.03 $\pm$ 5.71	1.40 $\pm$ 0.12	4.2 $\pm$ 4.1	1.05 $\pm$ 0.05	5.3( $\pm$ 3.1) $\times$ 10 <sup>6</sup>	6.74 $\pm$ 0.2
13	Control	10.25 $\pm$ 7.92	1.63 $\pm$ 0.47	43.8 $\pm$ 17.0	0.75 $\pm$ 0.0	8.4(1.5) $\times$ 10 <sup>6</sup>	7.2 $\pm$ 0.0
	Ozonated	42.68 $\pm$ 1.92	2.75 $\pm$ 1.79	2.4 $\pm$ 0.4	0.89 $\pm$ 0.0	22.25( $\pm$ 20.9) $\times$ 10 <sup>6</sup>	7.1 $\pm$ 0.2
15	Control	33.44 $\pm$ 1.24	1.73 $\pm$ 0.14	12.0 $\pm$ 7.8	0.62 $\pm$ 0.13	22.1( $\pm$ 4.7) $\times$ 10 <sup>6</sup>	7.1 $\pm$ 0.1
	Ozonated	37.42 $\pm$ 9.81	1.02 $\pm$ 0.26	0.8 $\pm$ 0.3	0.80 $\pm$ 0.13	43.0( $\pm$ 25.5) $\times$ 10 <sup>6</sup>	6.9 $\pm$ 0.0



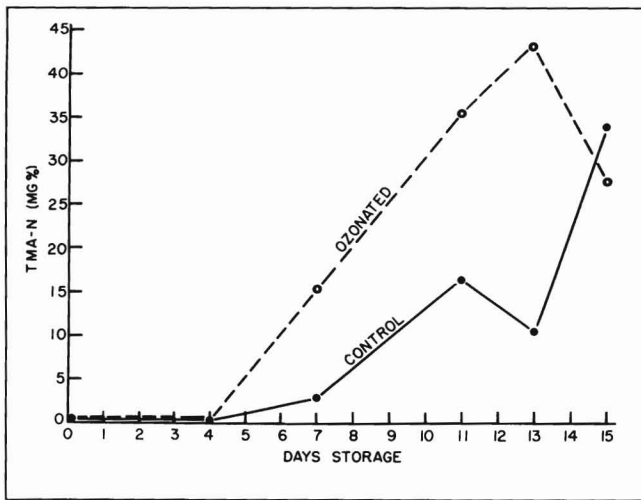


Figure 4.—Trimethylamine content of gutted cod held 7 days in aerated (control) or ozonated chilled seawater and then stored in ice.

if TMA formation began earlier in the ozonated fish.

Figure 6 shows supporting evidence with higher bacterial levels in the ozonated fish. At the beginning of this study, the bacterial level of ozonated CSW was 260,000 compared with 333,000 in the aerated CSW. The very small reduction in the original bacteria count may have been the cause of the unexpected results in Part II of this study. However, when the ozone concentration of the ozonated CSW was analyzed and found to be about 5 ppm, we assumed the treatment to be sufficient. Daily determination of the ozone concentration in the CSW immediately following ozonation was attempted, but the bloody, discolored nature of the water made ozone measurement uncertain. On day 7, when the remaining fish were removed from the CSW for storage on ordinary ice, the average bacterial count of the water from the ozonated tanks was  $34 \times 10^6$ /ml.

We are unaware of any reported studies of ozonated sea water as a preservation technique; however, Haraguchi et al. (1969) investigated the preservation of jack mackerel, *Trachurus trachurus*, and striped mackerel, *Caranx mertensi*, using ozone in a 3 percent NaCl solution. Although they reported an ozone concentration of only 0.6 ppm, it might be that their contact time between the fish and the ozone exceeded ours, since they

ozonated their brine solutions 30-50 minutes before the fish were added and an additional 30-60 minutes after the fish were added. The reported stability of ozone in their brine solution, however, is puzzling. The ozone may have been reacting with one of the components of the seawater to produce a bactericidal ion or compound.

They found initially, that the viable count of bacteria on the surface of the fish dropped to  $1 \times 10^{-2}$  to  $1 \times 10^{-3}$  of the control due to ozone treatment and was delayed about 4 days while decreases in sensory values (raw) were delayed by over one week, lengthening the storage life of the fish 1.2-1.6 times. Their results show, however, that the ozone treatment had little effect on the bacterial count on the muscle of the striped

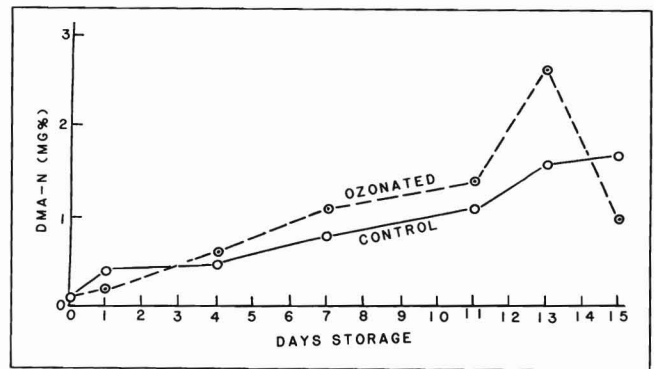
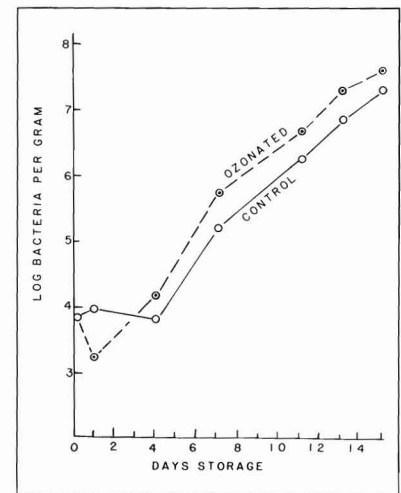


Figure 5.—Dimethylamine content of gutted cod held 7 days in aerated (control) or ozonated chilled seawater, and then stored in ice.

Figure 6.—Aerobic plate count of gutted cod held 7 days in aerated (control) or ozonated chilled seawater, and then stored in ice.



mackerel.

The major disadvantage due to the ozone treatment found by the Japanese investigators was the loss of the fresh-fish smell and the acquisition of a "dried-fish odor" which they attributed to the oxidation of the fish oil by ozone. This would be contrary to results of the Alaska salmon study (Nelson, 1982) where the level of rancidity in the control samples was more than 3 times greater than in the ozone treated samples.

In Part II of our study, as in Part I, rancidity was never detected by taste panelists and TBA numbers remained low (1.0) throughout the storage period. Treatment had no effect on pH changes and those values reported here are normal for iced cod.

The failure of ozone by various tech-

niques to prolong the shelf life of gutted cod is similar to the result of a study conducted by Vyncke (1981) which showed no extension in the shelf life of cod fillets by an ozonated water dip.

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