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The Effect of Aureomycin* Chlortetracycline on Fish Freshness

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WITH THE PRESENT DAY system of food handling and storage, a large part of the consumer market cannot hope to purchase fish within one day of its catch. Our vast system of marketing with its processors, terminal markets, and dealers, as well as long distance hauling has made improved methods of storage of food a vital necessity. The ever-increasing market, more distant from the sea, has made the process of keeping fish as fresh as possible almost as important as the catch itself. To date, the delivery of fresh fish has depended on ice or refrigeration to maintain this freshness. Now the need has become apparent for a means of holding that freshness longer. The purpose of this paper will be to present an answer to this need.

Tarr and co-workers (Tarr, 1944; Tarr and Deas, 1948; Bissett and Tarr, 1952; Tarr, Southcott and Bissett, 1952; Boyd Brumwell and Tarr, 1953; Tarr, Boyd and Bissett, 1954) for a number of years have been investigating additives for increasing the storage life of cod and salmon. In 1952 they reported success with antibiotics and particular success with chlortetracycline. They found that the bacterial spoilage of whole eviscerated fish was retarded significantly by chilling with ice containing one to four ppm of chlortetracycline by holding fish six days at —1°C in sea water containing three ppm chlortetracycline, or by a brief immersion in a 50 to 100 ppm chlortetracycline solution prior to icing. Tarr further found that chlortetracycline is fairly stable in flesh at low temperature but is readily destroyed upon heating. More recently Farber (1954) described the action of chlortetracycline on a number of fish and shellfish.

Deatherage and co-workers (Cahill, et al, 1952; Lepovetsky, et al, 1952; Goldberg, et al, 1953; Weiser, et al, 1953; Weiser, et al, 1954) have reported that the infusion of chlortetracycline inhibits bacterial growth in beef, while Kohler et al (1955) have shown a similar control in poultry.

One of the most perplexing problems associated with the determination of fish freshness is a reliable laboratory test. For our purposes microbial counts as well as organoleptic observations of odor appeared best. Experiments were conducted to establish a standardized procedure for this test.

Tarr reported microbial counts obtained by incubating for 24 hours at 10°C a one-inch center cut steak of the fish.

*Trademark of American Cyanamid Company for the antibiotic chlortetracyline is Aureomycin.

Table 1 shows the results of microbial counts made on mackerel purchased at a local store. One-inch center cut steaks were blended with three parts sterile distilled water. A duplicate steak was incubated 24 hours at 10°C prior to blending. Appropriate dilutions were made and bacterial counts were carried out on Difco Nutrient Agar, Difco Nutrient Agar prepared with sea water and ZoBell's Marine Agar (ZoBell, 1946). Counts were read after a 48 hour period at 25°C.

The data indicates that:

1. non-marine contamination predominates.
2. higher counts followed incubation technique.

The standardized procedure adopted for this work was to blend a one-inch center cut steak with three parts sterile distilled water. Appropriate dilutions were made and plated out on Difco Nutrient Agar. Plates were read after 48 hours at 25° C.

TABLE 1—MACKEREL

INITIAL MICROORGANISMS x 10^6 PER GRAM FISH

Incubation Period	Nutrient Agar	Sea Water Agar	Marine Agar
None	0.031	0.009	0.008
None	0.009	0.006	0.0004
24 Hours	0.043	0.010	0.0024
24 Hours	0.037	0.007	0.0008
5 DAYS STORAGE MICROORGANISMS x 10^6 PER GRAM FISH			
None	11.1		
None	3.7		
24 Hours	184.0		
24 Hours	78.0		

The method of application of chlortetracycline to fish depends on the usual mode of handling. It may be by chlortetracycline ice for those fish which are usually iced round or gutted. A chlortetracycline dip might fit best those fish that are usually handled with a rinse or pre-chill. A freezing brine containing chlortetracycline would suit best in the instance where fish are frozen by the immersion process.

An experiment was conducted to determine the uptake of chlortetracycline in fish treated with chlortetracycline. Whole butterfish and scrod steaks were treated by a 10 minute dip in fresh or sea water solution containing 10 ppm chlortetracycline. Samples were also frozen in a 22 per cent brine with 10 ppm chlortetracycline held at —12°C.

The chlortetracycline residue of the fish was determined by pad plate assay (Grady and Williams, 1953) measuring the inhibition of the material against *B. cereus* No. 5. An extract of the sample was prepared by blending five g in 25 ml of an acid acetone mixture composed of acetone 650 ml, distilled water 333 ml, and HCl 17 ml. Paper discs were dipped in the extracts and air dried for two to three hours at room temperature. The dried discs were laid on flat bottom Petri dishes containing 10 ml of Brain Heart Infusion Agar inoculated with *B. cereus*. After incubation the inhibitory zones were measured and the values calculated from the standard curve ranging from five to 0.025 mcg/ml chlortetracycline.

The data in Table 2 indicate that scrod steaks or whole butterfish may be treated effectively by sea or fresh water immersion or in a freezing brine containing chlortetracycline.

TABLE 2

SCROD STEAK AND WHOLE BUTTERFISH

Fish	Treatment	Chlortetracycline mcg/g Fish
Scrod steak	CTC 10 ppm tap water	5.5
Scrod steak	CTC 10 ppm sea water	4.5
Scrod steak	Freezing brine 22% + CTC 10 ppm	3.5
Whole butterfish	CTC 10 ppm tap water	1.33
Whole butterfish	CTC 10 ppm sea water	0.4
Whole butterfish	Freezing brine 22% + CTC 10 ppm	0.82

Ice containing five ppm chlortetracycline was prepared according to the method described by Upham et al, 1955. A number of species of fish were treated with chlortetracycline ice or with a dip in combination with chlortetracycline ice and the residual level determined.

TABLE 3

UPTAKE OF CHLORTETRACYCLINE IN MCG/G FISH

Fish	Treatment Dip	Ice	Steak	Flesh
Sea Bass	None	CTC	0.43	0.28
Sea Bass	5 ppm sea H_2O	CTC	1.55	0.25
Butterfish	None	CTC	0.54	0.38
Butterfish	5 ppm sea H_2O	CTC	0.98	1.1
Porgy	None	CTC	0.5	0.31
Porgy	5 ppm sea H_2O	CTC	1.58	0.25
Weakfish	None	CTC	0.05	0.7
Weakfish	10 ppm sea H_2O	CTC	1.6	0.37
Weakfish	10 ppm tap H_2O	CTC	0.88	0.18
Croaker	None	CTC	0.30	0.38
Croaker	10 ppm sea H_2O	CTC	0.30	0.20
Croaker	10 ppm tap H_2O	CTC	0.26	0.16
Scrod, Boston	None	CTC	2.4	-
Scrod, New Bedford	None	CTC	0.91	-
Salmon	None	CTC	-	0.09
Halibut	None	CTC	-	0.8

The data in Table 3 indicate that fish do take up chlortetracycline.

A series of tests were run on a variety of species of fish from a number of geographical locations to determine if an extension of storage life could be obtained.

Laboratory specimens of sea bass were obtained off the coast of New Jersey. Samples were packed in chlortetracycline ice, regular ice, and samples were also dipped 30 minutes in a five ppm chlortetracycline sea water solution prior to packing in chlortetracycline ice. After delivery to the laboratory, samples were held in the refrigerator until the bacterial counts were carried out by the standard procedure as outlined before.

TABLE 4—SEA BASS

TREATMENT		TOTAL MICROORGANISMS x 10^6 PER GRAM FISH		
Dip (5 ppm)	Ice	4th Day	7th Day	11th Day
None	Regular	980.0	8200 A	17,000 A
None	CTC	28.0	1700 C	11,000 A
CTC	CTC	1.4	770	840 A

A - putrid
B - strong odor
C - medium odor
D - slight odor

The data in Table 4 indicate that a chlortetracycline dip followed by chlortetracycline ice was the most effective preservative treatment. Although the storage life was extended beyond that of the control by chlortetracycline treatment, it should be pointed out that these samples were stored in a refrigerator not in the presence of chlortetracycline.

Samples of weakfish and croakers were dipped and iced on another trip off the New Jersey coast. The dips were prepared using 10 ppm chlortetracycline in both sea and fresh water. The water was drawn early and permitted to stand on deck until a uniform temperature of 15°C was reached. The fish were dipped 20 minutes and then iced in individual containers with chlortetracycline ice. In addition, individual containers of fish were iced under chlortetracycline ice or regular ice and held in this manner for the entire storage period.

The effectiveness of chlortetracycline in extending the storage of weakfish and croakers was clearly shown in this experiment. The data in Table 5 indicate that:

1. The storage life of weakfish is extended to the 15th day by chlortetracycline ice over the control life of eight days;
2. The extension of the storage life from 8th to the 15th day by a dip followed by chlortetracycline ice.

TABLE 5—WEAKFISH

TREATMENT		TOTAL MICROORGANISMS x 10^6 PER GRAM FISH					
Dip (10 ppm)	Ice	1 Day	4 Days	8 Days	11 Days	15 Days	18 Days
None	Regular	0.013	0.35	14.0 D	132.0 C	452.0 B	636.0 B
None	CTC	0.038	0.14	1.7	5.4	15.6 C	58.4 C
Sea H_2O	CTC	0.032	0.10	0.6	2.7	12.4 D	64.0 C
Fresh H_2O	CTC	0.054	0.51	0.5	3.2	15.2 D	80.8 C

B - strong odor
C - medium odor
D - slight odor

The data in Table 6 show that:

1. The decay in croakers was retarded from the 8th to the 15th day by chlortetracycline ice alone or in combination with the dip;
2. No detectable difference by odor or microbiological count between fish dipped in sea water or fresh water preparations of chlortetracycline.

TABLE 6—CROAKER

TREATMENT		TOTAL MICROORGANISMS x 10^6 PER GRAM FISH					
Dip (10 ppm)	Ice	1 Day	8 Days	15 Days	18 Days	21 Days	25 Days
None	Regular	0.064	5.6	240.0	320.0	320.0	552.0
None	CTC	0.032	1.4	23.2	39.6	61.6	56.0
Sea water	CTC	0.040	0.852	15.6	10.5	47.2	128.0
Fresh water	CTC	0.021	0.788	11.8	10.9	28.8	43.0

Samples of scrod taken out of New Bedford, Massachusetts, were iced under chlortetracycline ice or regular ice on shipboard. At the dock the samples were frozen and sent to the laboratory. After thawing they were kept on their respective ices for the test period. Storage time was taken as the days under ice not frozen.

The data in Table 7 demonstrate that chlortetracycline ice was effective in retarding the decay of scrod as evidenced by organoleptic findings and bacterial counts from the 22nd day to the 36th.

TABLE 7—SCROD (New Bedford)

TREATMENT	TOTAL MICROORGANISMS x 10^6 PER GRAM FISH				
Ice	8 Days	15 Days	22 Days	29 Days	36 Days
Regular	0.012	8.4	14.4 D	520.0 C	884.0 A
CTC	0.003	0.03	2.6	20.8	30.8 D

A - putrid
B - strong odor
C - medium odor
D - slight odor

Scrod obtained out of Boston, Massachusetts, showed a similar pattern.

TABLE 8—SCROD (Boston)

TREATMENT	TOTAL MICROORGANISMS x 10^6 PER GRAM FISH					
Ice	12 Days	16 Days	19 Days	22 Days	25 Days	28 Days
Regular	0.23	3.6	20.0 D	102.0 B	484.0 A	-
CTC	0.006	0.021	0.044	0.96	7.5	45.2

A - putrid
B - strong odor
C - medium odor
D - slight odor

The data in Table 8 indicate that chlortetracycline ice extended the storage life from the 19th to the 28th day as evidenced by odor and microbiological count.

Troll caught king salmon and halibut were obtained at Neah Bay, Washington. After evisceration, some fish were iced with chlortetracycline ice and others with regular ice and shipped to the laboratory under commercial handling conditions. The fish were trucked to Seattle and then shipped to New York by rail. They were in transit 13 days with one re-icing on the 6th day. The effectiveness of chlortetracycline ice under commercial handling conditions was clearly shown in this experiment.

The results obtained by organoleptic findings and bacterial counts shown in Table 9 indicate that decay in salmon was retarded by chlortetracycline ice to the 20th day over the control at 13 days. The salmon on regular ice upon receipt in the laboratory had a slight off-odor and was lighter in color with brown discolorations throughout. The salmon on chlortetracycline ice had a fresh sweet odor and a uniform pink color.

TABLE 9—SALMON

TREATMENT	TOTAL MICROORGANISMS x 10^6 PER GRAM FISH				
Ice	13 Days	16 Days	20 Days	22 Days	26 Days
Regular	28.4 D	91.6 C	424.0 A	712.0 A	
CTC	0.59	0.74	4.8 D	40.8 C	73.2 B

A - putrid
B - strong odor
C - medium odor
D - slight odor

The halibut handled in the same shipment had on arrival a strong odor throughout the entire fish and the flesh felt weak and soft. The chlortetracycline iced halibut had a slight off-odor in the gill cavity only. The flesh was firm.

TABLE 10—HALIBUT

TREATMENT	TOTAL MICROORGANISMS x 10^6 PER GRAM FISH				
Ice	13 Days	16 Days	20 Days	22 Days	26 Days
Regular	32.4 B	95.6 B	652.0 A	920.0 A	
CTC	0.12	0.15	3.0 D	69.2 C	108.0 A

A - putrid
B - strong odor
C - medium odor
D - slight odor

The bacterial counts shown in Table 10 indicated that decay was extended to the 20th day from a time previous to the 13th day as the controls were unacceptable when received by the laboratory on the 13th day.

Summary:

It has been clearly demonstrated by organoleptic observations and microbial counts that chlortetracycline is effective in prolonging the shelf life of fresh fish. The chlortetracycline may be applied as an ice, a dip, or a freezing brine. It is obvious that the choice of treatment is best determined by the present handling conditions. This, then, is offered as a means of keeping the quality of freshness so direly needed for our increasing market farther from the sea.

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The Use of Indole as an Indicator of Spoilage in Fresh Shrimp

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SOME OBJECTIVE TEST of shrimp quality is urgently needed by the industry to assist buyers and to help in the establishment of quality standards, and in the proper application of regulatory laws. One of the tests used has been to measure the quantity of indole produced during storage periods.

Tryptophane, an amino acid which is produced by the catalytic action of the enzyme tryptose upon the hydrolysis of albuminous matter is subject to various changes which eventually yield indole, among other substances.