EXPLORATORY FISHING AND TECHNOLOGY SESSION

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Chairman—JOHN MEHOS, *Liberty Fish and Oyster Company, Galveston, Texas*

Some Plant-Scale Applications of Chlortetracycline For Fish Preservation

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Introduction

THE AUTHORS PREVIOUSLY have shown (Farber, 1954; Farber and Lerke, 1956; Lerke and Farber, 1956, 1956-57) that the treatment in the laboratory of fillets, of whole fish, and of shrimp with the tetracyclines markedly extended their keeping quality at 42°F or lower. The question then arose as to the efficacy of the antibiotic treatment of fish under the more practical operating conditions existing in the fish plants. The present report presents the results obtained from some storage tests carried out in various fish plants in Monterey and San Francisco, including, in one case, the added effect of truck transportation from Monterey to and from San Pedro.

Experimental

Fish used — Fillets of sole (of various types, including English, Dover, Lemon and Petrale) and of various rockfish species (*Sebastodes* sp.) were taken from the filleting line in the plants for treatment and storage. The whole sole, sand dabs and mixed flat fish, including flounder, were taken from the trawl, which was dragged along the ocean floor, placed in fish boxes and covered with plain or treated crushed ice.

Treatments — The fillets were dipped five minutes in a five per cent salt brine with or without 5 or 10 p.p.m. of chlortetracycline (CTC) pure or as the mixture called Acronize PD. The whole fish on shipboard were placed in plain ice or in ice containing 10 p.p.m. of CTC as Acronize BI. On landing, the fish in the ice were either taken to the fish plant and stored without any further treatment (usually about six to eight hours) until brought to the laboratory, or they were picked up from the fishing vessel and taken directly to the laboratory.

Storage conditions — The fillets were stored in the usual metal shipping boxes in the plant cold storage rooms, where the temperatures ranged from 30° to 35°F. In one case the fillets in the metal boxes were covered with ice in wooden fish boxes and stored at 30°F for a few days before and after being shipped by truck from Monterey to San Pedro and return. The whole fish, after removal from the ice at the laboratory, were examined, judged, and then stored in the fish boxes, under damp sacks to minimize dehydration, at atmospheric temperature ranging from 55° to 60°F.

Laboratory examinations — The fish fillets or in the round were periodically examined organoleptically, chemically for their content of volatile reducing substances (V.R.S.), total volatile and trimethylamine nitrogen (T.V.N. and T.M.N.) and bacteriologically for their aerobic bacterial count, expressed as log of bacterial numbers. The procedures for these tests have been described previously (Lerke and Farber, 1956-57).

Results

An experiment was set up to test the effect on the keeping quality of rockfish fillets (*Sebastodes* sp.) treated with CTC under rather severe practical conditions. The fillets, taken from the filleting line of a Monterey fish plant, were dipped for five minutes in a five per cent salt brine and in a five per cent salt brine containing five p.p.m. of CTC. The two lots of fish were then placed in individual metal fish containers, which were then imbedded in ice in a wooden fish box and stored overnight at 30°F. The next day the boxes were filled with ice, closed and shipped by truck to San Pedro. They arrived there the night before a holiday. As a result, the boxes of fish remained outside the plant for about 36 hours at atmospheric temperature, that is from Wednesday evening July 3 to Friday morning July 5. The boxes were then re-iced, stored at 30°F over the weekend, and Monday morning were sent back by truck to Monterey, where they arrived that evening. They were stored overnight at 30°F and then sent in ice by truck to the laboratory in San Francisco for examination. The results of this test are summarized in Tables 1 and 2.

TABLE 1

STORAGE EXPERIMENT ON ROCKFISH FILLETS

Tues., July 2—Fillets dipped 5 min. in 5% NaCl brine with and without 5 ppm CTC, then placed in metal box which was covered with ice and stored at 30°F in Monterey.

Wed., July 3—Box in ice shipped by truck to San Pedro, where it arrived that evening.

Thurs., July 4—Holiday. Fish left outside of plant.

Fri., July 5—Fish taken into plant, re-iced and stored at 30°F.

Mon., July 8—Box re-iced and shipped by truck to Monterey, arriving that evening. Stored overnight at 30°F.

Tues., July 9—Fish in ice shipped to laboratory in San Francisco.

A storage test in a second fish plant is summarized in Table 3. Fillets of sole, removed from the filleting table, were dipped five minutes in five per cent salt solution and in five per cent salt solution containing 10 p.p.m. of CTC (as Acronize PD). Both lots were then stored in metal fish containers at 30°F in the cold storage room of the plant. Fillets were removed periodically for organoleptic, chemical and bacteriological examinations.

Another test in a third fish plant was carried out on sole fillets removed from the filleting table. They were also dipped for five minutes in a five per cent salt solution and in a five per cent salt solution containing 10 p.p.m. of CTC. The fillets of both lots were then stored in metal fish boxes in the cold room of the plant which was maintained at a rather fluctuating temperature of about 35°F. The fillets had a content of CTC ranging between 0.68 and 1.04 gamma

TABLE 2

STORAGE EXPERIMENT ON ROCKFISH FILLETS

Sample	*VRS*	*mg Volatile N/100 ml Total Trimethylamine*		*Log bacterial numbers*	*Condition*
Original	3.1	0.7	0	4.92	good
Control-after week	55.0	43.5	13.2	9.32	putrid
CTC treated-after week	12.1	6.6	3.9	8.81	V. sl. storage odor, still considered merchantable though not first quality
CTC content of fillets — O.224 gamma/gram					

per gram, averaging 0.87. The results of this storage test are summarized in Table 4.

Another storage test in the third plant was carried out on fillets of sole. These were treated as those in Table 4. The data obtained in this experiment are shown in Table 5. The CTC content of the fillets ranged from 0.30 to 1.32, averaging 0.70 gamma per gram.

Three experiments were carried out on whole fish on board two different fishing vessels. Table 6 summarizes the findings of the storage test from one of the boats. The fish when removed from the trawl on shipboard were placed in two wooden fish boxes, one containing plain ice and the other containing ice with 10 p.p.m. CTC (made with Acronize BI). The fish were brought ashore in the boxes of ice and taken to the laboratory after about 24 hours

TABLE 3

STORAGE EXPERIMENT ON SOLE FILLETS AT 30°F

Days Stored	*VRS*		*TVN*		*TMN*		*Log Bacteria*		*Remarks*	
	C	*CTC*	*C*	*CTC*	*C*	*CTC*	*C*	*CTC*	*C*	*CTC*
0	9.2		2.1		0		5.06		good	
1	6.2	7.6	1.5	1.5	0	0	4.95	4.75	"	
2	7.0	5.9	1.2	0.9	0	0	5.04	4.67	"	
4	5.4	5.6	2.5	1.5	0	0	5.60	4.58	"	
6	11.9	8.1	5.0	3.7	0.9	0	6.18	4.70	passable	good
8	11.8	10.2	7.8	5.0	3.7	0.6	6.65	4.82	"	"
9.5	15.6	5.4	8.7	5.3	6.8	0	7.72	4.95	stale and slimey	"
11	30.2	7.5	13.8	8.7	7.8	0	8.08	5.48	spoiled	"
13	—	5.9	—	—	—	0	—	5.85	—	"
15	—	5.4	—	5.0	—	0	—	6.18	—	"
17	—	3.8	—	5.6	—	0	—	6.10	—	"
18	—	4.5	—	5.6	—	0	—	6.15	—	"
20	—	9.7	—	7.8	—	1.9	—	8.70	—	passable
23	—	30.8	—	6.3	—	2.5	—	8.48	—	ammoniacal and discolored —not passable

TABLE 4

STORAGE EXPERIMENT ON SOLE FILLETS AT 35°F

Days Stored	*VRS* Cont.	CTC	*TVN* C	CTC	*TMN* C	CTC	*Log Bacteria* C	CTC	*Condition* C	CTC
0	3.9		2.0		0		5.72		good	
4	6.6	8.3	5.9	5.2	0.3	0	7.18	4.48	good	good
5	39.6	7.4	10.5	3.3	6.6	0	8.85	6.32	spoiled	good
6	—	2.2	—	2.6	—	0	—	6.40	—	"
7	—	3.0	—	3.3	—	0	—	6.96	—	"
8	—	2.6	—	3.3	—	0	—	8.04	—	"
11	—	11.8	—	6.6	—	4.6	—	8.96	—	passable
13	—	8.8	—	8.6	—	3.9	—	9.00	—	passable
15	—	5.9	—	2.6	—	0	—	8.92	—	"
16	—	27.2	—	5.3	—	3.3	—	9.00	—	spoiled

TABLE 5

STORAGE EXPERIMENTS ON SOLE FILLETS

Days Stored at 35°F	*V.R.S.* Control	CTC	*TVN* C	CTC	*TMN* C	CTC	*Log Bacteria* C	CTC	*Condition* C	CTC
0	1.6		0.9		0		6.08		good	
2	4.9	1.3	1.5	0.6	0	0	6.05	5.91	good	good
5	2.7	2.7	0.9	0.6	0	0	6.36	5.60	good	good
7	6.5	5.9	1.2	0.9	0.3	0	7.73	6.18	"	good
8	13.5	—	4.1	—	0.6	—	7.85	—	near borderline	—
9	40.0	6.6	6.0	0.9	3.1	0	8.79	7.60	spoiled	good
12	—	11.3	—	3.2	—	0.3	—	—	—	passable
13	—	7.5	—	1.2	—	0	—	—	—	good
14	—	15.1	—	6.2	—	1.2	—	—	—	borderline passability
15	—	44.5	—	3.1	—	2.5	—	8.30	—	spoiled

from the time they were caught. They were removed from the ice and stored in the same fish boxes at atmospheric temperature of 55° to 60°F.

The results shown in Tables 7 and 8 were obtained from the same fishing boat on two different occasions. The fish of Table 7 were whole sand dabs (*Citharichthys sordidus*) which had been treated similarly to those of Table 6; that is, they were placed in plain ice and in ice containing 10 p.p.m. CTC on removal from the trawl on shipboard. On arrival at the laboratory before storage at ambient outside temperatures they were between 18 and 24 hours out of the water. The fish of Table 8 were mixed flat fish, consisting of sole, dabs and flounders (*Platichthys stellatus*). They were treated aboard ship as those of Tables 6, 7 and 8. On arrival at the laboratory they were about 24 hours out of the water. They were also removed from the ice and stored at ambient outside temperatures between 55° and 60°F. All the whole fish were filleted and the skinned fillets were then analyzed.

TABLE 6

STORAGE EXPERIMENT ON ROUND SOLE

Hours	V.R.S.		TVN		TMN		Log Bacteria		Remarks	
Stored at 55-60°F.	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated
0	3.3	2.2	1.9	0.6	0.6	0	4.66	4.00	good more slimey	good
30	14.2	4.9	5.0	1.2	1.2	0	—	—	borderline	good
48	33.5	6.6	8.2	1.6	2.5	0.3	7.49	5.42	spoiled	good
56	—	7.4	—	4.4	—	0.12	—	6.08		good

TABLE 7

STORAGE EXPERIMENT ON WHOLE SAND DABS

Hours	V.R.S.		TVN		TMN		Log Bacteria		Remarks	
Stored at 55-60°F.	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated
0	7.1	6.0	1.9	1.5	0	0	5.86	5.34	fairly good more slimey	good
24	7.1	3.3	4.4	3.4	0.6	0	5.70	3.85	same	same
32	14.6	6.2	7.9	4.2	3.1	0	6.85	6.00	some off odor to fish more slimey & discolored	same
48	47.2	12.1	12.5	6.2	6.9	0.6	7.72	7.45	spoiled	still passable appearance

TABLE 8

STORAGE EXPERIMENT ON ROUND FLAT FISH

Hours	V.R.S.		TVN		TMN		Log Bacteria		Remarks	
Stored at 55-60°F.	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated	Plain ice treated	CTC ice treated
0	3.8	3.8	3.1	3.1	0	0	5.28	4.74	good more slimey	good
18	18.1	4.4	3.8	2.4	1.9	0	6.98	4.50	some off odor to whole fish, very slimey	good
23	32.9	6.9	6.4	3.4	3.1	0	6.61	5.28	spoiled	good
40	—	3.3	—	2.5	—	0	—	6.83		still passable
48	—	8.7	—	3.1	—	0.9	—	7.60		slight off odor to whole fish

Discussion

The data of Tables 1 and 2 show that even under rather adverse handling conditions a CTC dip prolonged the keeping quality of rockfish fillets by about a week. This probably represents a minimum value, with longer keeping times possible under more favorable storage conditions.

From an examination of the data of Tables 3, 4, and 5 a number of observations can be made.

1. A CTC dip definitely prolongs the storage life of sole fillets.

2. The actual length of the added storage time resulting from the CTC dip depends upon many factors, including, among others, the history of the whole fish from catching to the start of filleting, the sanitation of the filleting operation, and the storage temperature of the fillets. As a possible example of the effect of the original bacterial load on the fillets, on their keeping quality, the data of Tables 3, 4 and 5 may be applicable. In Table 9 are summarized the original bacterial counts of the fillets and the subsequent estimated time in days before any spoilage became evident. It will be seen that for the untreated fillets the original bacterial load did not apparently have too much effect on their keeping quality. For the CTC-treated fillets, on the other hand, there was an apparent increase in keeping time with a decrease in initial bacterial load. The difference in storage temperatures may not have too much effect in this case, since the fish were stored in commercial cold storage rooms in the plants, which were opened many times a day for varying times and the temperatures fluctuated in all cases. Therefore, even though the lower average storage temperature no doubt contributed to the longer keeping time, the differences in bacterial loads very likely also could have had an effect. This also accentuated once more the marked interrelationship between sanitary operating conditions and the beneficial effect of an antibiotic treatment.

3. The content of V.R.S. was found to be a more useful guide to the condition of the fillets than any of the other chemical and bacteriological criteria. In this connection it may be of interest to point out the lack of correlation between the total aerobic bacterial count and the condition of the fillets, as determined either organoleptically or chemically by the V.R.S. content. Total bacterial counts higher than in the spoiled controls were found in CTC-treated fillets which were judged still passable. When methods become available to determine the number of potentially spoilage bacteria their count may correlate better with their condition. This bacteriological problem is at present being investigated in this laboratory.

The experiments on the effect of storage of whole flat bottom fish in plain and CTC-containing ice were of interest. Apparently, the treatment of the whole fish immediately on removal from the water with the antibiotic in the ice must pre-

TABLE 9

CORRELATION BETWEEN ORIGINAL BACTERIAL COUNT OF SOLE FILLETS AND SUBSEQUENT STORAGE TIME BEFORE BEGINNING OF SPOILAGE

Log Initial Bacterial Count	Days before any spoilage evident	
	Control untreated	CTC-dipped
5.06	9	21.5
5.72	4.5	15.5
6.08	7.5	13.5

vent the initial development of the spoilage organisms. As a result, subsequent longer storage periods of the treated whole fish without any ice was possible, compared to fish originally placed in plain ice. The fact that the flat fish have a very small intestinal tract area, compared to the total area of the fish, also must have added a favorable influence on the storage periods. Other experiments carried out on small round fish, such as herring and sardines, which have a relatively large intestinal tract area, have shown that a more prolonged contact with the antibiotic during the storage period is apparently necessary to obtain any significant prolongation of keeping quality. The effect of the intestinal tract area is even more exaggerated in such large fish as tuna, where spoilage inside the intestinal tract becomes of increasing importance, in addition to that starting on the outside.

The average uptake of CTC by the fillets was 0.598 gamma per gram, with a range of 0.224-1.32 gamma per gram.

Summing up all the observations made, it can be said that a treatment with an antibiotic, such as chlortetracycline (CTC), has a definite beneficial effect on the subsequent keeping quality of rockfish and sole fillets and of round flat fish, including sole species, dabs and flounders. The extent of the prolongation of the keeping quality was dependent, among other factors, on the storage temperature, the original bacterial load and history of the fish from time of catch to that of landing ashore.

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REFERENCES

FARBER, LIONEL

1954. Antibiotics as Aids in Fish Preservation. I. Studies on Fish Fillets and Shrimp. Food Technol. *8*, 503.

FARBER, LIONEL and PETER LERKE

1956. The Effect of Chlortetracycline on Shrimp Storage at Various Temperatures. Proc. Ninth Annual Meeting Gulf and Caribbean Fisheries Inst. Nassau, Bahamas, p 26.

LERKE, PETER and LIONEL FARBER

1956. Antibiotics as Aids in Fish Preservation. II. Further Studies on Fish Fillets. Proc. F.A.O. Symp. on Fish Processing, Rotterdam, Netherlands, 25-29, June, paper No. 2.

1956-57. Prolongation of the Keeping Quality of Fish and Shellfish by Antibiotics. Antibiotics Annual, 966.