

CHEMICAL PRESERVATIVES IN FOODSTUFFS

IV. PROLONGATION OF THE KEEPING QUALITY OF FRESH FISH BY ANTIBIOTICS

REINO R. LINKO, OLAVI E. NIKKILÄ and JORMA J. LAINE

The State Institute for Technical Research, Helsinki

Received March 29, 1961

Of the three main types of spoilage that occur in fish — bacterial, oxidative and enzymatic — the first named is of the greatest importance, as it causes economic losses to the fisherman, the fish industry and the consumer. Fish is an ideal medium for the growth of bacteria, and it is a very perishable food. Psychrophilic bacteria which originate from water usually form the natural microflora of fish. In the main, bacteria invade the originally sterile flesh of fish from the surface slime through the skin, but invasion also takes place through the gills and viscera.

The use of antibiotics is not confined only to the cure of human and animal disease, but in many countries investigators have evinced great interest in the use of antibiotics as preservatives for food.

In Finland, NIKKILÄ (11) has studied the effect of antiseptic washing on Baltic herring, and LINKO (10) has suggested that antibiotics should be used to increase the storage time of fresh fish, as for example is done in the U.S.A., Canada, Japan and Spain.

The use of antibiotics for food stuffs was tried out for the first time in 1943 when experiments with penicillin were carried out in the General Laboratories of the American Can Company's Research Division (12). TARR and his co-workers in Canada were the first to realize the potential value of antibiotics as preservatives for fresh food, and they reported the results of their first efforts in 1944 (22). Subsequently (26) they reported the findings of their studies on the preservation of fish and meat by means of streptomycin, penicillin, subtilin, polymyxin B, circulin, neomycin, bacitracin, gramicidin, metholyl gramicidin, tyrothricin, rimocidin, terramycin, chloromycetin, aureomycin, and one unnamed antibiotic. It was found that only three of these antibiotics, i.e. aureomycin (chlortetracycline), terramycin (oxytetracycline) and chloromycetin had effect on the preservation of both fish and beef at temperatures of 0°—21°C. Some other investigators (5, 7, 8, 13, 20, 31) also concluded that only broad-spectrum antibiotics are useful for extending the freshness of food.

In fish preservation the tetracyclines, tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC) are the most effective antibiotics (9). According to TARR (23, 24), CTC is always more effective than OTC, but some other investigations (6, 20) have not confirmed these claims, although other data confirm the superiority of both OTC and CTC over TC (9).

To achieve the best results, treatment with antibiotics should be started immediately the fish have been caught (14, 31). The antibiotic can be applied to the ice used for fish storage, as a solution in dips or sprays for the whole fish prior to icing or after filleting (7) or by way of addition to the chilled brine or sea water used in place of ice (19, 24).

SHEWAN (15) observed that preservation of whole gutted white fish (*Gadus merlangus*) in ice containing 5 ppm of aureomycin extended the storage life of the fish, and the point of inedibility was reached 7 to 10 days later than was the case with fish in ice without antibiotics. According to SHEWAN (15), ALBERTSON (1), and KREUZER (8), the effect of the antibiotic in iced fish does not become apparent until after the 10th to 14th day of storage.

SHEWAN and STEWART (17) stored godling (*Gadus minutus*) and haddock (*Gadus aeglefinus*) immediately after catching in ice containing 5 ppm CTC. Organoleptically, the most striking feature in the raw fish was the suppression of thick yellow slime after storage in ice for more than 16 days. Fillets from the antibiotic-treated fish always looked more translucent and less bleached, whitish and opaque than did the control fillets. Tests carried out with fish from both laboratory and pilot-scale trials showed that antibiotic-treated fish could be used for all the purposes for which iced fish are usually used.

VELANKAR and KAMASTRY (30) stored different species of fish for 27 days in ice containing 5 ppm of CTC. In these experiments they obtained that the use of CTC is not of advantage if the storage lasts only one week. However, the effect of CTC becomes apparent on longer storage. Psychrophilic bacteria appear to a very reduced extent in CTC-ice-stored fish. For the assessment of Indian fish, determination is necessary of both trimethylamine and bacterial counts.

A substantial extension in the storage life of Pacific round herring (*Etrumeus micropus*) (27) resulted after treatment by storage in sea water containing ice and 10 ppm of CTC on the boat, by storage in ice containing the antibiotic 5 ppm after landing, or by their combination. The storage with the antibiotic in sea water and in the ice prolonged the storage life approximately 90% at 15° to 20°C, and by at least 40% at -1° — +2°C.

The best results were obtained by employing the antibiotic treatment immediately after catching, in connection with the chilling (3, 13, 18). Not only the keeping quality but also the fresh taste of the fish are then maintained for a much longer period (31).

In dip treatment the concentration of the antibiotic has in general been higher, usually 10 to 25 ppm of CTC and OTC, the dipping time varying from 1 to 60 minutes before storage at temperatures from 0° to 15°C; the increase in the storage time amounted to 40 to 100% (16). TOMIYAMA et al. (28) found that dipping sardines (*Sardinella melanosticta*) for 30 minutes or longer in 5% brine containing 10 to

20 ppm CTC produced a preservative effect as judged by organoleptic or volatile base-N tests.

Other investigators (21) have noted that at levels of 5, 10 and 20 ppm in brines at temperatures of 0°, 4.5° and 10°C OTC was very effective in preserving the freshness of sockeye salmon (*Oncorhynchus nerka*). CTC was as effective as OTC. Spraying salmon with OTC solutions of 20 and 100 ppm antibiotic also prolonged the freshness of salmon in storage at room temperature.

SHEWAN (15), using 5 ppm of CTC in the ice, found that CTC was always present in fish skin, but could not be detected in the flesh until fish had been stored for 9 to 12 days. After 28 days, CTC was detected mostly in the skin, less than 1 ppm being usually found just beneath the skin, and none at all in the flesh 2.5 cm or more below the surface.

TARR (24) has carried out a number of experiments concerned with the penetration of antibiotics into fish muscle, and their destruction during heating. In experiments made with gray cod (*Gadus macrocephalus*) it was noted that when the fish was stored in ice containing 1 ppm CTC, there was a rather slight penetration after several days storage. Fish stored in refrigerated sea water containing CTC absorbed the antibiotic more easily than did the iced fish.

TARR (24) continues that the tests have indicated rather rapid destruction of CTC on heating. Results indicate that the heating of fish to internal temperatures of between 82° and 99°C will destroy from about 80 to 90 per cent of added CTC (fish flesh containing 6.5 and 16 ppm of the antibiotic). Similar results have been obtained by TOMIYAMA, YONE and MIKAJIRI (29) and BISSET and TARR (4).

In the present study an investigation was made into the effect of antibiotic treatment on the keeping quality of the Baltic herring, the most important fish in Finland.

Methods

The material consisted of fresh Baltic herring (*Clupea harengus var. membranus*) in the rigor mortis state. The experiments were carried out with muscle homogenates, fillets and whole fish.

The antibiotics used were Acronize 40 (active ingredient: chlortetracycline sulphate 40 %), Acronize 10 (active ingredient: chlortetracycline hydrochloride 10 %), Biostat GP (active ingredient: oxytetracycline hydrochloride 21.6 %), Acronize BI (active ingredient: chlortetracycline hydrochloride 16.5 %) and Biostat X—AI (active ingredient: oxytetracycline hydrochloride 8.5 %.) The first three of those mentioned were used in experiments concerned with muscle homogenates and dipping treatments, the last two in experiments with antibiotic ice.

The samples were kept at 2° and 10°C, and the bacteria cultured at room temperature. The total bacterial counts were computed from nutrient agar, and calculated per gram of sample. In addition to the bacteriological study, organoleptic evaluation was also made.

Experiments with the muscle homogenate. Skinless fillets were used in these experiments. For each sample, 100 g of fillets were aseptically weighed into 600 ml of sterile water. The tissue was homogenized by means of a Turmix mixer. The homogenate was divided in sterile conical flasks, and 1, 5, 10, 25 and 50 ppm of the above-mentioned active antibiotics in sterile water added. A sample of homogenate served as control, a corresponding quantity of sterile water being added. At intervals, samples were taken from the homogenates for total bacterial counts on nutrient agar.

Experiments with antibiotic ice. Ice flakes were produced in an ice flaking machine. Ordinary ice flakes were used as control. The antibiotic ice contained 5 ppm of the active ingredient.

Fish, packed in ice flakes, were kept on perforated aluminium trays. The surfaces on the fish were completely separated from each other. The temperature of storage was 2°C, and ice flakes were added when necessary.

For the purpose of bacteriological study, samples were taken from the fish muscle in accordance with the method of Aschehoug and Vesterhus (2).

Dipping of fish in antibiotic solutions. In preliminary experiments, dipping times of 1, 2, 5, 15, 30 and 60 minutes were tried out, and 1 min. proved to be the most appropriate. In the experiments proper the samples were dipped for 1 min. in the antibiotic solutions, and the control sample for a corresponding period in sterile water. The concentration of antibiotic in the experiments was 50 and 100 ppm of the active ingredient.

The experiments with whole fish were carried out at 10°C, and those with skinless fillets at 2°C. The samples were kept in plastic bags during the experiments.

Samples were taken from the fish muscle for total bacterial count in the same way as in the experiments with antibiotic ice.

Results

Experiments with muscle homogenate. Tables 1 and 2 illustrate the dependence of the spoilage and bacterial content of muscle homogenates, prepared from fresh Baltic herring fillets, on the concentration of the antibiotic, and the time at 2°C (Table 1) and 10°C (Table 2).

The results showed that at 2°C (Table 1) the bacterial count of the control samples without antibiotic increased rather rapidly. Even after 4 days a weak off-odor was observable; after 8 days it was very strong. By contrast, 1 ppm of oxytetracycline or chlortetracycline was able completely to prevent the spoilage of the homogenate for 4 days. When the concentration of the antibiotic was increased, the bacterial counts decreased steadily becoming even lower than the original level. The growth-inhibiting effect of antibiotic in bacteria was most clearly shown after 8 days. Whereas the control samples contained more than 2 million bacteria per gram of homogenate, the samples containing 1 ppm of chlortetracycline

Table 1. The effect of oxy- and chlortetracyclines on the viable bacteria content and odor in muscle homogenate of Baltic herring stored at 2°C.

Storage time days	OTC ppm	Micro-organisms $\times 10^6$ per gram	Organoleptic observations	CTC ppm	Micro-organisms $\times 10^6$ per gram	Organoleptic observations
0	0	0.0025	No off-odor	0	0.0025	No off-odor
4	0	0.048	Slight odor	0	0.048	Slight odor
	1	0.011	No off-odor	1	0.0023	No off-odor
	5	0.0014	—	5	0.0007	—
	10	0.0005	—	10	0.0005	—
	25	0.004	—	25	0.0005	—
	50	0	—	50	0.0018	—
8	0	2.2	Strong odor	0	2.2	Strong odor
	1	0.052	Medium odor	1	0.020	Medium odor
	5	0.01	Slight odor	5	0.008	Slight odor
	10	0.004	—	10	0.005	—
	25	0.002	—	25	0.001	—
	50	0.02	—	50	0.0004	—
12	0	500	Strong odor	0	500	Strong odor
	1	132	—	1	7.08	Medium odor
	5	7.41	Medium odor	5	2.76	—
	10	0.36	Slight odor	10	0.027	Slight odor
	25	0.81	—	25	0.026	—
19	0	>1000	Putrid	0	>1000	Putrid
	1	912	—	1	684	—
	5	798	—	5	23	Strong odor
	10	125	—	10	37	—
	25	5.2	Strong odor	25	6.7	—
	50	9.0	—	50	1.7	—

Table 2. The effect of oxy- and chlortetracyclines on the viable bacteria content and odor in muscle homogenate of Baltic herring stored at 10°C.

Storage time days	OTC ppm	Micro-organisms $\times 10^6$ per gram	Organoleptic observations	CTC ppm	Micro-organisms $\times 10^6$ per gram	Organoleptic observations
0	0	0.0025	No off-odor	0	0.0025	No off-odor
4	0	5.7	Medium odor	0	5.7	Medium odor
	1	12.3	—	1	0.52	Slight odor
	5	0.72	Slight odor	5	0.27	—
	10	0.41	—	10	0.024	No off-odor
	25	0.42	—	25	0.005	—
	50	0.09	No off-odor	50	0.003	—
8	0	440	Putrid	0	440	Putrid
	1	510	—	1	314	—
	5	239	—	5	520	—
	10	5.2	Medium odor	10	91.2	Strong odor
	25	1.1	—	25	31.4	Medium odor

or oxytetracycline had only 20,000 and 52,000 bacteria. When the antibiotic concentration was 25 ppm, the total bacterial count was maintained on a level corresponding to that at the beginning of the experiment. After storage for 12 days, 10 ppm of antibiotic still inhibited the spoilage of the muscle homogenate, but after 19 days all the samples were spoiled.

At a temperature of 10°C (Table 2), the reproduction of the bacteria was so rapid that already after four days the control samples were clearly spoiled. However, 10–50 ppm of chlortetracycline and 50 ppm of oxytetracycline completely inhibited the spoilage of homogenates. After 8 days, however, all the homogenates containing antibiotic were also spoiled.

Experiments with antibiotic ice. The bacterial content of fish kept in antibiotic ice and in ordinary ice (control) evidenced at first no particular differences. This is demonstrated in Fig. 1, where the bacterial counts of muscle after 8 days were still rather low, varying in different cases from 100 to 130/g. Subsequently the

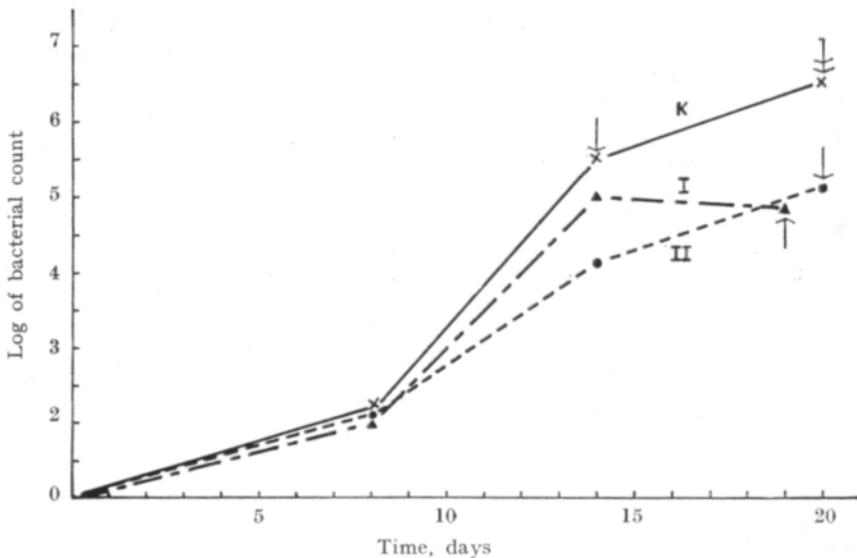


Fig. 1. The growth of bacteria in Baltic herring stored in ice with and without antibiotic.

K ordinary ice (control)

I CTC-ice 5 ppm

II OTC-ice 5 ppm

↓ beginning spoilage

↓↓ completely spoiled

bacterial content of muscle began to rise clearly, and after 14 days definite differences were observed in fish treated in different ways.

The amount of bacteria was 500,000/g in the control, 100,000/g in samples kept in CTC-ice, and correspondingly 20,000/g in samples kept in OTC-ice. Between 14 and 20 days of storage, the amount of bacteria continued to rise in the control

sample, whereas in samples kept in antibiotic ice it decreased. On comparison with the control the difference was then quite definite. The bacteria in the control amounted to 5 millions/g and in OTC-ice to 200,000/g after 20 days, and in CTC-ice 90,000/g after 19 days.

In organoleptical tests, the spoilage was observable after 1 week's storage, by which time the control was clearly spoiled, whereas fish kept in antibiotic ice were in almost perfect condition.

Dipping of fish in antibiotic solutions. The results of the dipping experiments showed that the different antibiotics used exhibited no obvious differences, but that the spoilage in the control was much more rapid than in samples treated with antibiotics (Figs. 2 and 3).

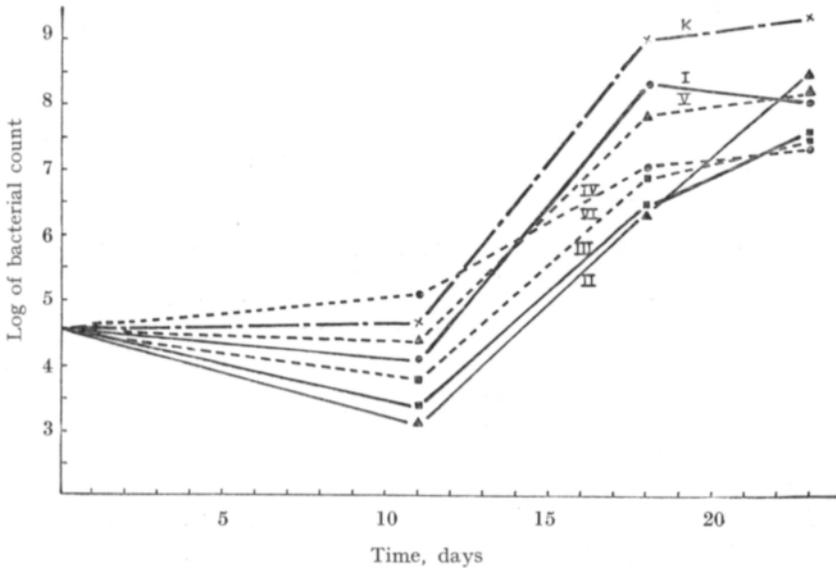


Fig. 2. The growth of bacteria in Baltic herring fillets dipped for 1 minute in antibiotic solutions and stored in plastic bags at 2°C.

K no antibiotic (control)	IV OTC 10 ppm
I CTC 10 ppm	V " 25 "
II " 25 "	VI " 50 "
III " 50 "	

It was noticeable that the amounts of bacteria in fish fillets kept at a storage temperature of 2°C decreased in all the samples dipped in antibiotic solutions other than in that dipped in solution containing 10 ppm of OTC (Fig. 2). After 11 days the quantities of bacteria began to rise in all samples, but not until after 14 days was the bacterial count of samples dipped in solutions containing 25 and 50 ppm of CTC (curves II and III) the same as at the beginning of the experiments. After

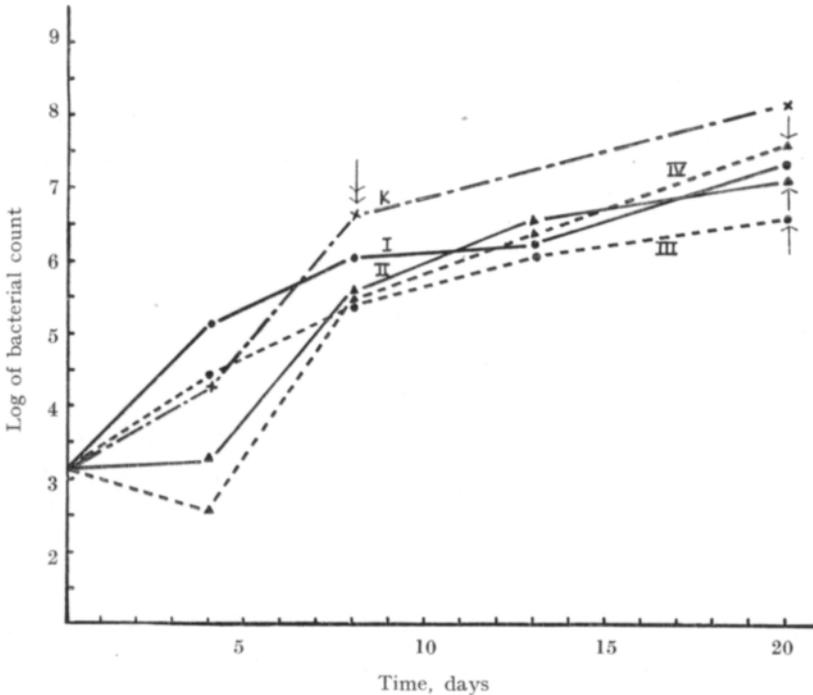


Fig. 3. The growth of bacteria in whole Baltic herring dipped for 1 minute in antibiotic solutions and stored in plastic bags at 10°C.

K	no antibiotic (control)	III	OTC 50 ppm
I	CTC 50 ppm	IV	100 ppm
II	100 ppm		
↓	beginning spoilage		
↓	completely spoiled		

18 days, the bacterial count in the antibiotic-treated sample varied from 2.3 to 130 millions/g, whereas in the control it was 1000 millions/g.

In experiments performed with whole fish at 10°C (Fig. 3), a decrease was also to be noted in 4 days in the bacterial amounts of the samples which had been dipped in solutions containing 100 ppm of antibiotic. By contrast, the quantity of bacteria in fish dipped in solutions containing 50 ppm of antibiotic first increased in a way very similar to the control, and differences were observable only after 8 days. In organoleptical tests, the control was definitely spoiled in 8 days, whereas samples dipped in antibiotic solutions were not spoiled until after 20 days.

Discussion

The bacteriological and organoleptical studies under laboratory conditions showed that the broad-spectrum antibiotics OTC and CTC have the effect of improving the keeping quality of fresh Baltic herring.

Contrary to some earlier observations (14), the above mentioned antibiotics were found to be bactericid even at very low concentrations; in muscle homogenates this was so at a concentration of 1 to 5 ppm (Table 1) and in fish fillets 10 ppm (Fig. 2). When for instance fish fillets, dipped for 1 min. in solutions containing 10 and 25 ppm of CTC, were kept for 11 days at 2°C, the quantity of bacteria per g of muscle decreased from 60,000 to 12,000 and 2,000, respectively.

In the experiments made with the muscle homogenate, it was possible to note the immediate effectivity of the antibiotics as compared with the control, and to follow the dependence of the bacterial growth on the concentration of the antibiotic, temperature, and time.

At a low temperature (2°C), 10 ppm of CTC or OTC inhibited bacterial growth for 8 days (Table 1). At a higher temperature (10°C) 50 ppm of antibiotic could inhibit bacterial growth for 4 days (Table 2). These results agree with earlier findings (4). On the other hand, divergent observations have also been made as regards the temperature. TARR, SOUTHCOTT and BISSET (25) found that quantities of CTC and OTC as low as 10—25 ppm almost completely inhibited bacterial growth in minced flesh of halibut (*Pleuronectes hippoglossus vulgaris*), Chinook (*Oncorhynchus tshawytscha*) and brill (*Rhombus laevis*) which had been stored at 30° and 37°C for periods of up to 10 days.

With whole Baltic herring the results proved to be somewhat different. In fish dipped in antibiotic solutions, differences in relation to the controls were found at the beginning of the experiment but only with high concentrations (100 ppm) of the antibiotic (Fig. 3, curves II and IV), whereas at lower concentrations such differences were observable only after 8 days (curves I and III). The situation was similar with fish stored in antibiotic ice, where differences in relation to the controls kept in ordinary ice were first observable after 14 days (Fig. 1). This lag phase may be due to the fact that the effect of antibiotics is focused on the bacteria in the surface slime of fish, and penetration through the skin into the muscle is at first rather slow.

The results of the organoleptic tests as indicated in Figs. 1 and 3 give information of the spoilage from another angle. In this respect it was possible to state from the sliming of the surface of fish, the intensity of odor, and the firmness of the muscles that, for instance, in the dipping experiments at 10°C, the untreated control fish were completely spoiled in 8 days, whereas the fish dipped in antibiotic solutions were not spoiled until after 20 days. Similarly, in fish kept in ordinary ice the spoilage began after 14 days, but in fish kept in antibiotic ice not until after 19—20 days.

From the practical point of view it is to be noted that the favorable effect of antibiotic ice does not become evident until the storage time of the fish is at least one week. When using antibiotic ice the temperature of the fish is maintained sufficiently low, and thus the temperature itself alone decelerates the growth of bacteria and increases the storage life of the fish, but at the same time the effectivity of the antibiotics also increases. The concentration of the antibiotic can thus be kept very low, max. 5 ppm.

Dipping treatments come into question in the employment of short storage

times; the concentration of the antibiotic must here be greater, from 50 to 100 ppm, depending on the temperature. This procedure is particularly applicable to fish fillets.

The studies made show that under the same conditions oxytetracycline and chlortetracycline are similar in their efficacy, and have a selective influence on the bacteria causing spoilage of fish. In this respect more information will become available as a result of experiments with bacterial strains isolated from fish.

Summary

A study has been made of the effect of antibiotics in the storage of fresh Baltic herring. Experiments were made with muscle homogenates, to which antibiotics were added, and whole fish and fillets, treated by means of dips in antibiotic solutions and storage in antibiotic ice. The temperatures studied were 2°C and 10°C. The antibiotics employed were chlortetracycline and oxytetracycline.

It was observed that the antibiotics improved the keeping quality of Baltic herring. In dipping treatments, and in experiments with muscle homogenates, the favorable effect of the antibiotic was observable even at the early phase of the storage, whereas when antibiotic ice was used such effect was only discovered after about one week's storage. In a comparison of chlortetracycline and oxytetracycline no essential differences were observed in their effectivity.

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SELOSTUS:

ELINTARVIKKEIDEN KEMIAALLISISTA SÄILÖNTÄAINEISTA. IV. TUOREEN KALAN SÄILYVYYDEN PARANTAMINEN ANTIBIOOTTIEN AVULLA

REINO R. LINKO, OLAVI E. NIKKILÄ ja JORMA J. LAINE

Valtion teknillinen tutkimuslaitos, Helsinki

Silakalla suoritettiin tutkimus antibioottien vaikutuksesta tuoreen kalan säilyvyyteen. Kokeita tehtiin lihashomogenaateilla, joihin lisättiin antibioottia, sekä kokonaisella kalalla ja fileillä, jotka kastettiin antibioottiliuoksiin tai säilytettiin antibioottijäissä. Säilytyslämpötilat olivat 2°C ja 10°C. Käytetyt antibiootit olivat klortetrasykliini ja oksitetrasykliini.

Kokeissa voitiin todeta, että antibioottien käyttö paransi silakan säilyvyyttä. Lihashomogenaattikokeissa ja antibioottiliuosta käytettäessä (»dipping»-käsittely) vaikutus tuli esille jo säilytyksen alkuvaiheessa, kun taas antibioottijäätä käytettäessä vaikutus oli todettavissa vasta n. yhden viikon säilytyksen jälkeen. Klortetrasykliinin ja oksitetrasykliinin tehokkuudessa ei todettu oleellisia eroja.