CHEMICAL PRESERVATIVES IN FOODSTUFFS

VI. The effect of silver ions on microbes, in particular on the flora of fresh fish

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Some metals possess what is known as an oligodynamic effect, i.e. they exert either a microbistatic or a microbicidic influence in very small concentrations. Silver is the most effective oligodynamic metal of all. It is assumed that the silver ions denature the protein part of the enzymes in the microbial cell. Silver possesses an oligodynamic effect irrespective of whether it derives from metallic silver or silver salt.

In laboratory experiments with *E. coli* as the test organism, Wuhrman and Zobrist (4) found that the bactericidic effect of silver depended primarily upon the silver ion concentration, but also on the pH, and the concentrations of Cl^-, PO_4^{-3} Ca^{+2}, and oxygen. On the other hand, it is claimed (2) that when silver is present in water at concentrations of 0.02 to 0.03 ppm, its bactericidal effect is 40 times greater than that of the usual chlorine treatment, without the unpleasant tastes and odours often found in heavily chlorinated water. In such concentrations, silver is not toxic to human beings and animals.

To date, the most extensive applications have been sterilization of water with the silver ion developed in Germany (2), Switzerland (4) and the U.S.A. (2) used by breweries in disinfecting the equipment and pipelines, in flour mills and in the sterilization of surgical instruments. In addition, research indicates (2) that silver treated water could be of benefit in food processing for washing products and containers, to give the product a longer shelf-life. It has even been suggested that ice or snow containing silver ions could be utilized for the preservation of fish or poultry and would provide more enduring protection against decomposition than ordinary ice does.

The present study has been concerned with examination of the microbicidic effect of silver ions upon different microbial cultures in water, and keeping the quality of fresh Baltic herring *(Clupea harengus* var. *membranus)* stored in ice containing silver ions. The purpose is to discover new applications which will aid the food industry to prevent microbial attacks in daily practice.

Methods

An Elektro Katadyn Apparatus, Type 1/4, was employed for preparation of water containing silver ions. In this apparatus, silver ions are produced by aid of electric current; their concentration being regulated by changing the current. Within the range 20 to 100 mA used there was found to be a linear relationship between current intensity and silver ion concentration. The equipment operated well throughout the experiment.

Tap water containing chlorine to the extent of 13.8 mg/l, with a calcium hardness of 4.0°, and a magnesium hardness of 1.2° (German degrees of hardness), was used throughout.

During a period of 24 hours, the loss of silver ions in water solutions kept in glass and polyethylene flasks in the dark was about 50 % with a 100 μg Ag^+/l solution, and 30 % with a 500 μg Ag^+/l solution. There were no marked differences between the glass and polyethylene containers.

Experiments with water containing silver ions. Escherichia coli, Saccharomyces cerevisiae, and a natural mixed culture isolated from unpasteurized milk were subjected to experiment. First, a homogeneous cell suspension was prepared and aliquots added to flasks containing sterile water. Following this, fresh stock solution of silver (500 μg Ag^+/l), was added to give final silver ion concentrations of 20, 40, 60, 80 and 100 μg Ag^+/l. Counts of viable microbes were made initially and during the experiment.

The initial values were 6.35×10^5/g for *E. coli*, 8.65×10^5/g for *S. cerevisiae*, and 7.85×10^5/g for the natural mixed culture.

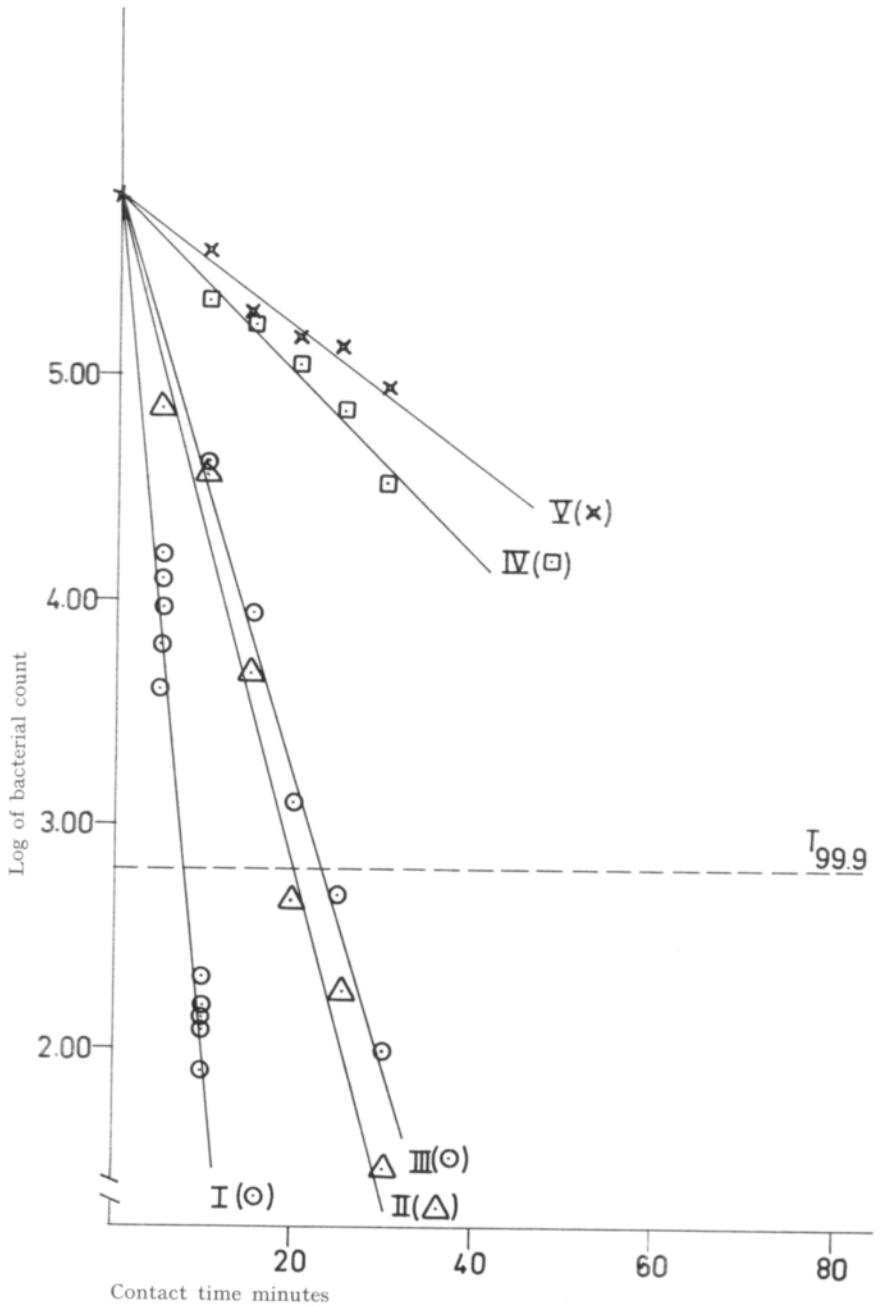
Removal of microbes with water containing silver ions. S. cerevisiae was utilized as the test organism in these experiments. 1/3 litre glass bottles were infected with a cell suspension of *S. cerevisiae*, spreading it evenly in the bottles, which were then drained, and filled with water containing silver ions. The concentrations were 100, 500 and 1000 μg Ag^+/l and the contact times 10, 20, 40 and 60 seconds. The bottles were then filled with sterile water, and counts of the remaining *S. cerevisiae* made. Bottles with sterile water alone served as controls.

Experiments on stainless steel pipes. S. cerevisiae and *E. coli* were the test organisms in these experiments, which were made with 6-metre metal pipes 3/4 in. in diameter. The pipe was first filled with the microbe suspension for 24 hours, and subsequently washed with sterile water. The quantity of water used in the washing was three times the capacity of the pipe. After this, the pipe was filled with water containing silver ions (500 μg Ag^+/l), and microbial counts were made at stated intervals.

Experiments with ice containing silver ions. Ice flakes were produced with a Scotsman 100 Ice Flaking machine. Silver was added beforehand to the water, which then contained 0, 50, 100, 200 and 400 μg Ag^+/l.

For each sample, fresh Baltic herring, 0.5 kg, was weighed out on a plastic tray. Each tray was covered with ice flakes, and the samples were stored at +4° C or +9° C. Each sample was re-covered with ice every 12 hours.

Bacteriological samples were taken by the method of LINKO et al. (3) in the beginning and after 1, 2, 4, 8, 12 and 16 days of storage. During the course of the experiment, completely spoiled samples were discarded.



— — — — — 99.9 % death rate
I = 100 μg Ag/l $T_{99.9}$ = 8 min
II = 80 » » = 20 »
III = 60 » » = 23 »
IV = 40 »
V = 20 »

Fig. 1. Death rate of *Escherichia coli* in water containing different concentrations of silver ions.

Determinations were made as follows: the total bacterial count on SPC- (Standard Plate Count) and fish-agar; the latter was prepared from Baltic herring. 1 kg fish fillets was cooked in 1 litre of water for 20 minutes, and then filtered through a sieve. An addition was made of 15 g of agar for 1 litre of the filtrate. MPN-technique was used for coliforms (1). In addition, organoleptic evaluation was performed, with the texture, the colour, the slime formation, and the odour of the fish as the criteria.

The incubation times in the bacteriological studies for total counts were 72 – 120 hours at 20° C; in the coliform procedure a temperature of 35° C was applied in all the experiments. In the coliform determination, the counts represent total coliforms, and not merely faecal coliforms.

Representative colonies were isolated from the petri dishes and pure cultures thus obtained. Seventy of these cultures were tested for gelatin liquefaction on nutrient gelatin, for the formation of acid or base on RV-agar, and for the formation of gas in nutrient broth. The cultures were also kept at + 4° C for four days on SPC-agar to detect the psychrophilic strains. Subsequently, the cultures were tested for the effect of silver at concentrations of 0, 50, 100, 200 and 400 μg Ag^+/l. The technique applied was the same as that in the experiments with water containing silver ions.

Results

Experiments with water containing silver ions. Figures 1, 2 and 3 illustrate the effect exercised by different silver ion concentrations upon the micro-organisms studied. $T_{99.9}$ was taken as the measure of the treatment; $T_{99.9}$ refers to the time after which 99.9 % of the original cell in the sample had been destroyed.

With *E. coli*, the $T_{99.9}$ was 8, 20 and 23 min. with silver ion concentrations of 100, 80 and 60 μg Ag^+/l, respectively (Figure 1). At silver ion concentrations of 20 and 40 μg Ag^+/l, the $T_{99.9}$ was longer, although these concentrations were still adequate to destroy *E. coli*.

With *S. cerevisiae* the $T_{99.9}$ was 5, 9 and 22 minutes with silver ion concentrations of 100 – 60, 40 and 20 μg Ag^+/l, respectively (Figure 2), this organism being more sensitive to silver ions than *E. coli*. The differences in the death rates within the range of 60 to 100 μg Ag^+/l were not detectable by the method applied.

With the natural mixed flora isolated from unpasteurized milk, the $T_{99.9}$ was obtained in 50, 57 and 71 minutes with silver ion concentrations of 100, 80 and 60 μg Ag^+/l, respectively (Figure 3). In these studies, the natural mixed culture was so resistant to silver that 40 and 20 μg Ag^+/l had only a slight effect upon it.

Removal of microbes with water containing silver ions. As *S. cerevisiae* is the most sensitive of the micro-organisms tested in experiments with water containing silver ions, it was selected for this experiment. It was found that microbial counts did not differ significantly from the control in any treatment other than that at 100 μg Ag^+/l with a contact time of 60 seconds, in which approximately 60 % of the yeasts originally present were killed.

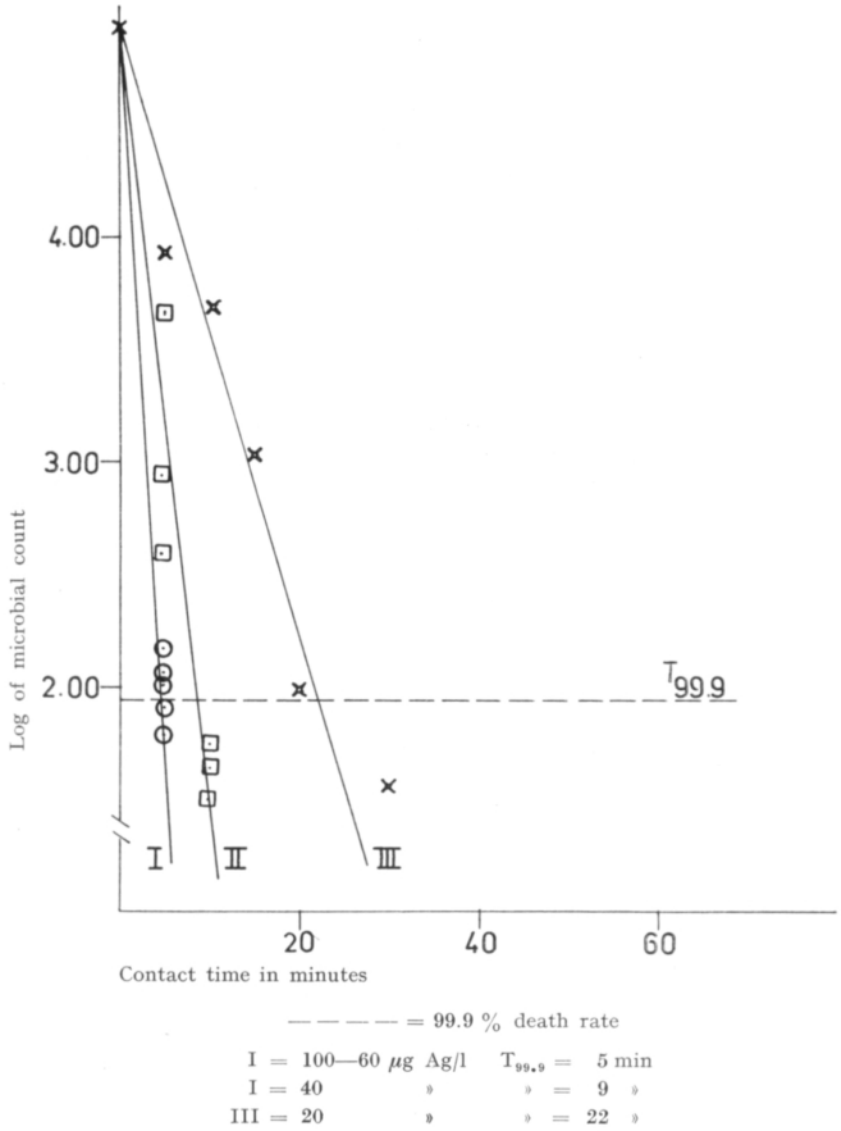


Fig. 2. Death rate of *Saccharomyces cerevisiae* in water containing different concentrations of silver ison.

Experiments in stainless steel pipes. Figures 4 and 5 indicate the death curves of *E. coli* and *S. cerevisiae.* The results once again show the yeast to be less resistant to the microbisidic action of silver than *E. coli.* The $T_{99.9}$ for *S. cerevisiae* was 28 minutes, whereas it took 8 hours with the *E. coli* to reach the same degree of destruction. The natural mixed culture was not affected by this treatment, no essential destruction observable 200 hours.

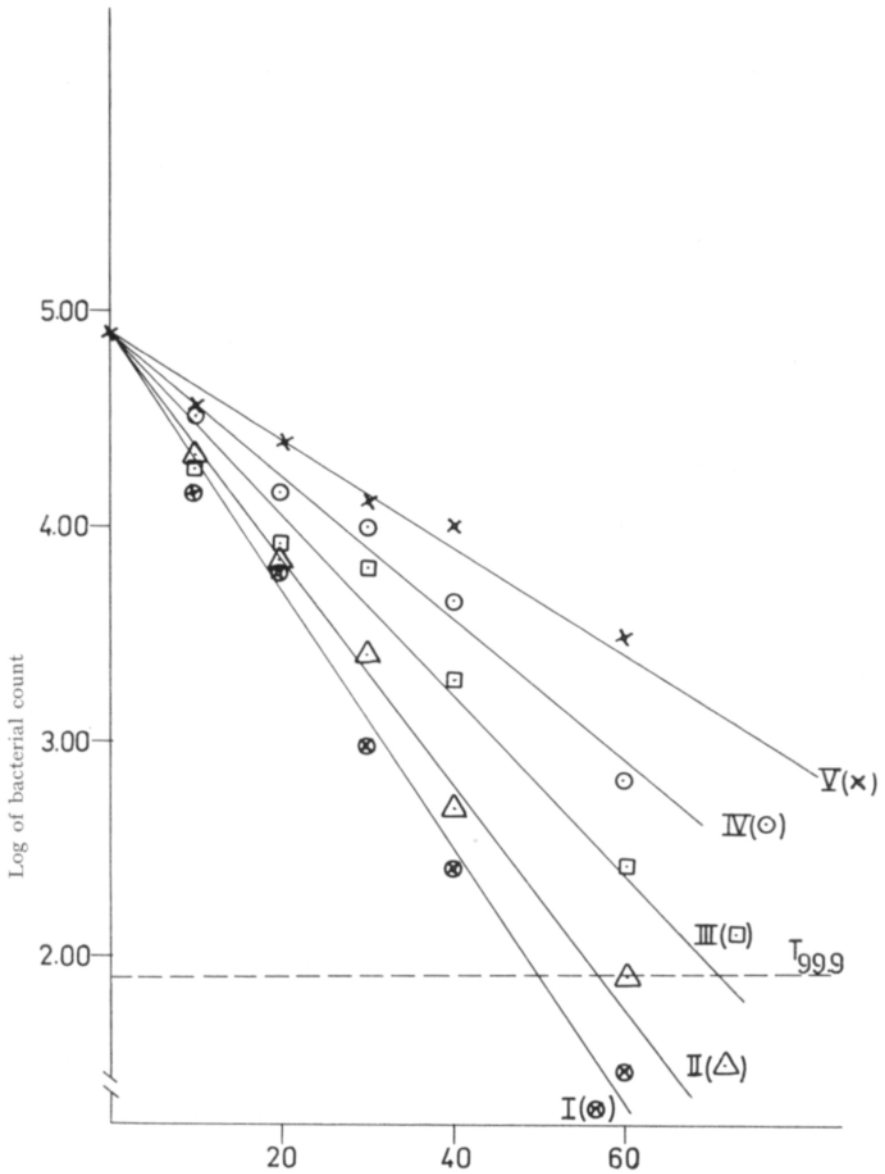


Fig. 3. Death rate of the natural mixed bacterial flora, isolated from unpasteurized milk in water containing different concentrations of silver ions.

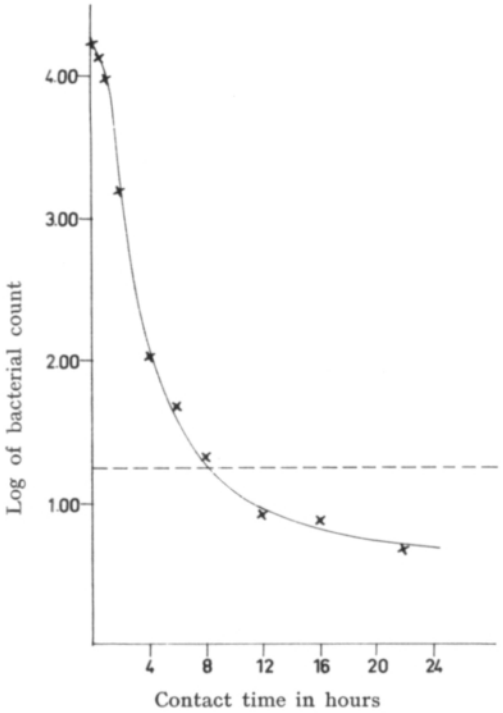


Fig. 4. Death rate of *Eschericia coli* in a stainless steel pipe at a silver ion concentration of 500 μg/l.

— — — — = 99.9 % death rate

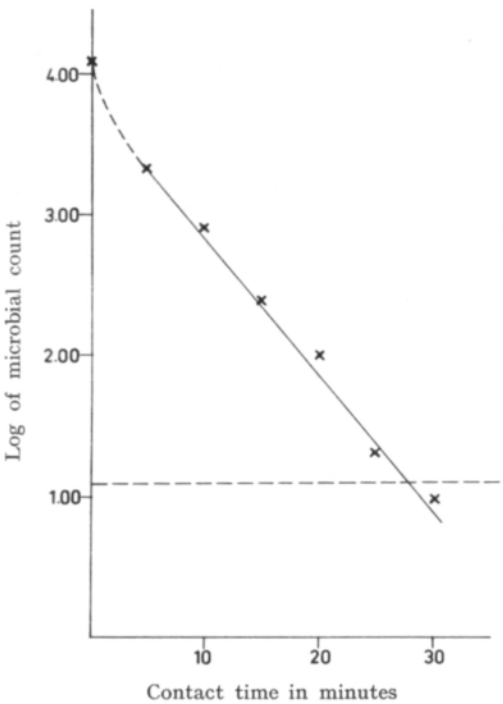


Fig. 5. Death rate of *Saccharomyces cerevisiae* in a stainless steel pipe at a silver ion concentration of 500 μg/l.

— — — — — = 99.9 % death rate

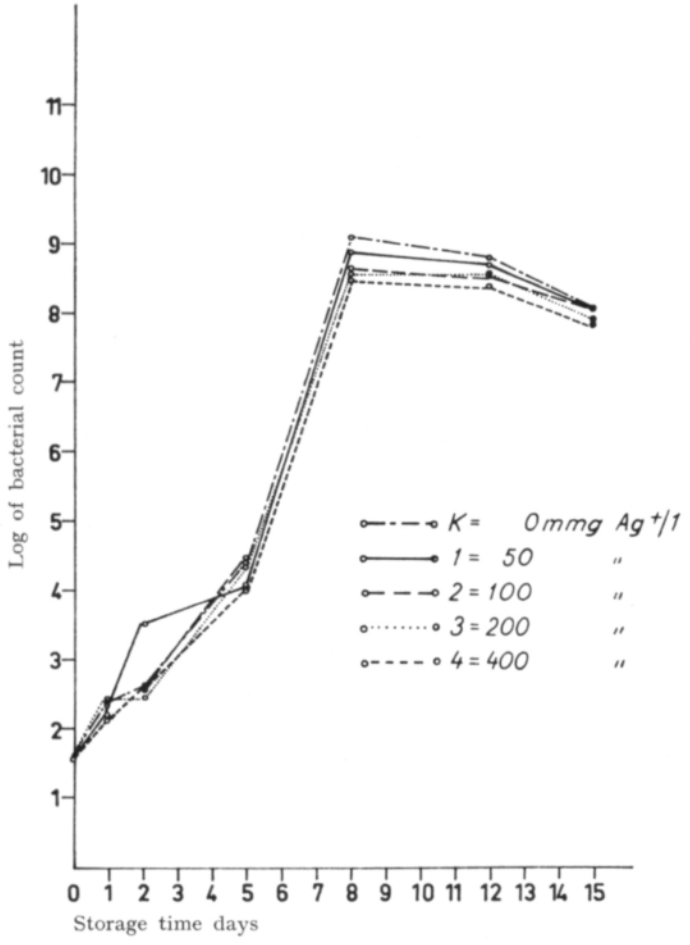


Fig. 6. Growth of bacteria on Baltic herring stored with ice containing silver ions at + 4° C.

The experiments indicated that the microbicidic action of the silver ions was reduced during the experiment. Microbes which resist longer times of contact could avoid the lethal effect of silver, as was the case with the natural mixed culture. With *E. coli* (Figure 4) the slope of the curve diminished rapidly after the 99.9 % destruction level had been reached. With a sensitive micro-organism such as *S. cerevisiae* (Figure 5), the destruction rate is so rapid that loss of the silver did not weaken the effect of the treatment.

Experiments with ice containing silver ions. Figures 6 and 7 indicate that the presence of silver ions in ice did not improve the keeping quality of Baltic herring at either +4° C or +9° C. Similar results were obtained from fish-agar. The difference in temperature played a much more important part in the experiment than the silver ion concentrations.

MPN for coliforms (Table 1) showed that there existed no correlation between the silver ion concentration of ice and the coliform counts.

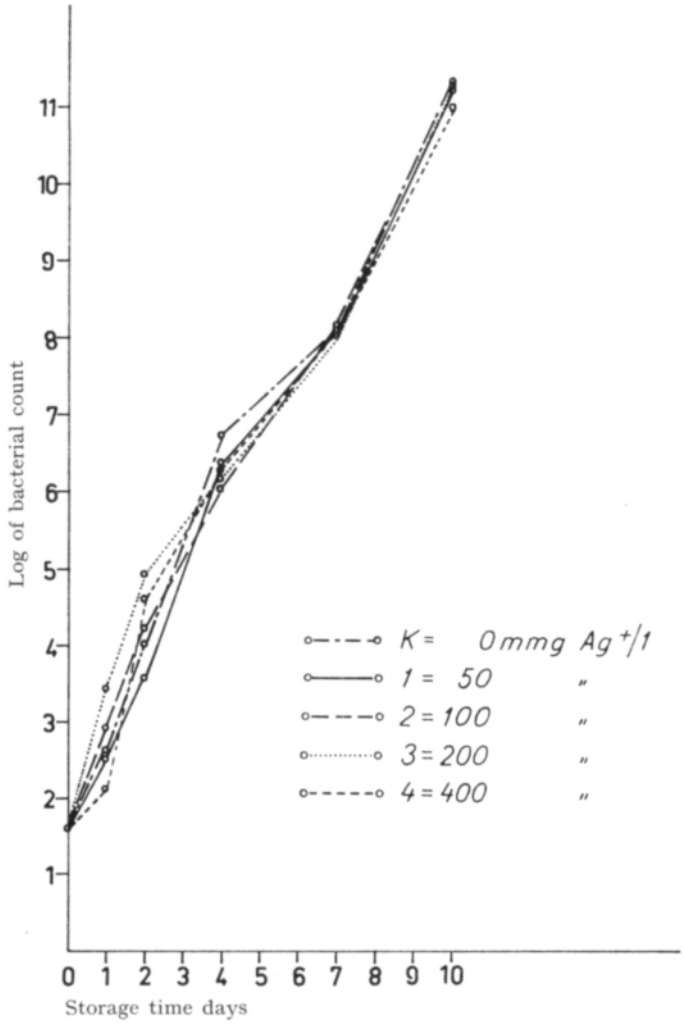


Fig. 7. Growth of bacteria on Baltic herring stored with ice containing silver ions at + 9° C.

It was found organoleptically that the fish spoiled at +4° C in 8 – 9 days, and at +9° C in 4 – 5 days. The presence of silver ions in ice did not significantly change this phenomenon, or extend the keeping quality.

In the experiments with the 70 pure cultures isolated from the Baltic herring, it was established that 65 of them were psychrophilic, and five were in the coliform group. In these experiments, very interesting results were obtained. While silver ions did not kill these organisms when they were living on the surface of the fish, these ions were very effective (Figure 8) against the same organisms in water. When treated with water containing 50, 100, 200 and 400 μg Ag^+/l for 48 hour at 21° C, a quantity as low as 50 μg Ag^+/l brought about a 99.9 % destruction. Coliforms were more sensitive than the other micro-organisms (Figure 8). All the 65 psychrophilic cultures liquefied gelatin and were base-forming; eight of them were gas-producing.

Table 1. MPN for coliforms isolated from fresh Baltic herring stored with ice containing silver ions.

Storage time days	Ag^+/l µg	Storage temperature + 4° C	+ 9° C	Storage time days	Ag^+/l µg	Storage temperature + 4° C	+ 9° C
0	0	2	2		100		>2400
1	0	4.5	0		200		4.5
	50	0	2		400		0
	100	0	0	8	0	23	
	200	0	0		50	2	
	400	0	0		100	0	
2	0	2	2		200	0	
	50	4.5	0		400	0	
	100	0	3.6	10	0		0
	200	2	0		50		2
	400	2	0		100		>2400
4	0		0		200		>2400
	50		14		400		1.8
	100		0	12	0	0	
	200		6.8		50	13	
	400		0		100	0	
5	0	6.8			200	1.8	
	50	49			400	0	
	100	2		15	0	23	
	200	7.8			50	33	
	400	11			100	0	
7	0		0		200	0	
	50		3.7		400	0	

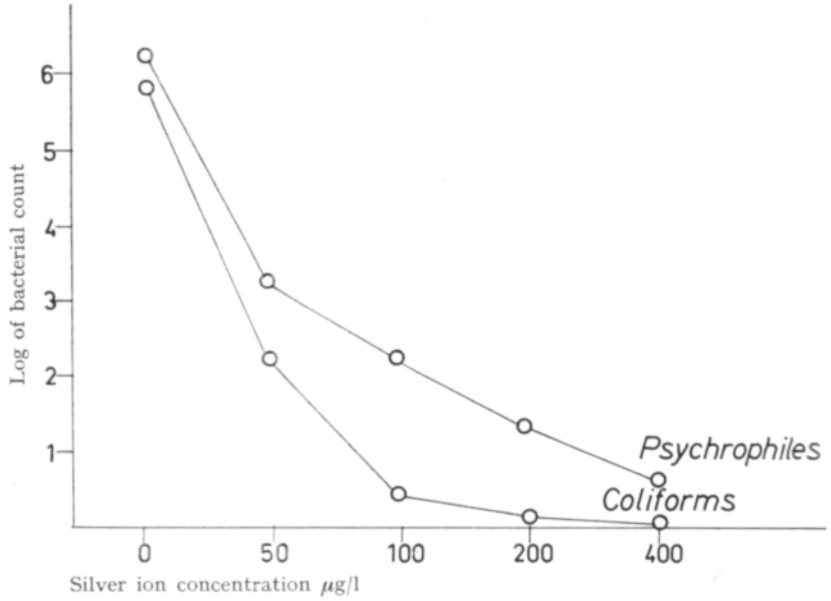


Fig. 8. Death rate of bacterial pure cultures isolated from Baltic herring in water containing different concentrations of silver ions. The psychrophile-curve is the average of the growth curves of 65 psychrophilic bacteria, and the coliform-curve is the average of the growth curves of 5 coliform types of bacteria.

Discussion

These studies served to disclose many interesting features regarding the effect of silver-ion treatment upon the microbes tested. In water containing silver, the effect was rapid and took place at low silver ion concentrations. However, it appeared that a natural mixed culture was more resistant to the microbicidic action of silver ions than were the pure cultures.

In the rinsing treatment, the silver ions were less effective than had been expected, requiring impracticably long contact times. Should silver ion treatment be used in washing machines for bottles, the times needed are too long. In practice, the contact times must be calculated in accord with the speed of the washing machines.

As regards the use of water containing silver ions in cleaning pipelines in food manufacture, the experiments gave positive results, although the natural mixed culture again appeared more resistant than the tested pure cultures to the microbicidic action of silver ion.

In experiments with ice containing silver ions being utilized for the improvement of the keeping quality of fresh fish, the results were negative. No marked bactericidal effect was found and the treated fish spoiled as quickly as did the controls. Nonetheless, it was interesting to note that microbes isolated from the spoiled fish and subjected to silver ions in water were killed in the same concentrations as those in which they survived on the surface of the fish.

It can accordingly be concluded that microbes themselves can be destroyed by silver ions in aqueous solutions, but that silver ions become less effective when the microbes are protected by a natural enviroment.

Summary

A study has been made of the effect of silver ions upon microbes in water, in rinsing and washing experiments, and in the storage of fresh Baltic herring in ice containing silver ions.

It was observed that silver ions were microbicidic in all experiments except in those with stored fish. Even in this case, silver ions were effective in killing bacteria when they were isolated and kept in an aqueous solution.

In all the experiments the pure cultures were less resistant than the natural mixed cultures.

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SELOSTUS:

ELINTARVIKKEIDEN KEMIALLISISTA SÄILÖNTÄAINEISTA

VI Hopeaionin vaikutus mikrobeihin ja erityisesti tuoreen kalan bakteeristoon

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Tutkittiin hopeaionin vaikutusta mikrobeihin käyttäen koeorganismeina *Escherichia colia, Saccharomyces cerevisiaeta* sekä pastöroimattomasta maidosta ja tuoreen kalan pintalimasta eristettyä luontaista bakteeriflooraa. Kokeissa todettiin hopeaionilla olevan voimakkaan mikrobisidisen vaikutuksen. Tätä vaikutusta heikensi kuitenkin orgaanisten aineiden läsnäolo. Myöskin luontainen sekakasvusto osoittautui vastustuskykyisemmäksi hopeaionin suhteen kuin samojen bakteerien puhdasviljelmät.

Tutkittaessa tuoreen kalan säilyvyyttä tavallisessa jäähileessä ja hopeaionipitoisesta vedestä tehdyssä jäähileessä todettiin niiden säilyttävän vaikutuksen olevan käytännöllisesti katsoen saman.