AN ABSTRACT OF THE THESIS OF

Donald John Pentzer for the M. S. in Hort. Products
(Name) (Degree) (Major)

Date Thesis presented May 8, 1936.

Title The Preservative Action of Ionic Silver on Fruit Juices

Redacted for privacy

Abstract Approved:
(Major Professor)

In an effort to develop a method of preserving fruit juices which would not change the color and taste of the fresh juice, this investigation with ionic silver was undertaken. A brief review of the literature on metallic sterilization is included. The author found that the kinds and numbers of organisms present, the active acidity of the juice, the clarity of the juice, and the temperature of storage are the important factors in preservation with ionic silver.

Pear and apple juices may be preserved with 1,000 to 2,000 gammas of silver per liter. Loganberry juice, because of its low pH, cannot be preserved readily with silver. It was found that the yeast cell is inhibited from growing when first subjected to silver ions, but that finally it is actually killed. Silver salts in solution are as effective as the silver produced by the electro-Katadyn method and does not affect the color of the juice as does the Katadyn silver. With all forms of silver investigated, a slight metallic taste was noted in concentrations as low as 1,000 gammas of silver per liter of juice.

THE PRESERVATIVE ACTION OF IONIC SILVER ON FRUIT JUICES

by

DONALD JOHN PENTZER

A THESIS

submitted to the

OREGON STATE AGRICULTURAL COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1936

APPROVED:

Redacted for privacy

Professor of Horticultural Products

In Charge of Major

Redacted for privacy

Head of Department of Horticulture

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Chairman of College Graduate Council.

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Professor Ernest H. Wiegand under whose direction the work was undertaken; to Dr. W. S. Brown for his criticism of the manuscript; to W. J. Miller, graduate student in Horticultural Products, for his suggestions during the investigations; and to the Katadyn Process Corporation of New York for loaning the electro-Katadyn equipment necessary for the investigations.

TABLE OF CONTENTS

	Page
Introduction	5
Review of the Literature	5
Discovery of Metallic Sterilization	5
Solubility of Silver	6
Development of Silvered Sands	7
Toxicity Due to Ions	8
Critical Concentrations	9
Characteristics of Germicidal Action	12
Ionic Silver and Higher Organisms	13
Procedure	15
Investigations	17
Apple Juice	17
Pear Juice	22
Loganberry Juice	27
Action of Silver on Yeast	29
Discussion of Results	30
Summary	37
Literature Cited	38

THE PRESERVATIVE ACTION OF IONIC SILVER ON FRUIT JUICES

INTRODUCTION

In the common practice of sterilizing fruit juices with heat, an abnormal flavor resembling a cooked or slightly scorched flavor often is detected in the product. Also, the color of the natural fruit juice often is altered when the juice is subjected to the treatment with heat. These alterations of flavor and color undoubtedly lower the saleability of the juice. If some method of sterilization of fruit juices could be found by which the original color, aroma, and taste of the natural fresh juice could be maintained, a product could be produced which would have an enormous sales appeal, and a large amount of the surplus fruit which, at the present time, has no market could be utilized in the form of juices for beverage purposes. With this view in mind, the following investigations were undertaken.

REVIEW OF THE LITERATURE

A review of the scientific literature has shown that a mass of fundamental research on metallic germicides has been accumulating for the past forty years.

Discovery of Metallic Sterilization. In 1893, a botanist by the name of Naegeli found that inconceivably small quantities of copper will hinder the growth in fresh water of

the filamentous alga Spirogyra, a common inhabitant of fresh water known also as pond scum. Naegli satisfied himself that the water became toxic to Spirogyra if a copper plate were merely immersed in it and then removed. Present day copper sulphate treatment of natural waters may date from this experiment. Effective quantities were so small that they could not be determined by the analytical methods available at the time of the discovery. To this phenomenon of minute quantities of substances has been given the term "oligodynamic" effect, which is derived from the Greek word meaning force of trifles or traces. Later work based on this phenomenon has explained the reaction considerably.

Solubility of Silver. Freundlich and Söllner (6) report that a silver plate when suspended in distilled water for several days goes into solution. As much as 2×10^{-5} grams of silver per liter is dissolved from a piece of silver 40 sq. cm. in size suspended in 100 c.c. of water for twenty-one days. Algae take up silver from such water and display an oligodynamic injury. Proof that the algae absorbed silver was made by a chemical analysis of the algae which indicated that one gram of dry algae so treated contained 5×10^{-1} grams of silver.

Wernicke, et al (41) also found that when strips of metallic silver were placed in water, enough silver was dissolved to give the water an oligodynamic property. They report that when the water and strip of metallic silver are

kept in an atmosphere of hydrogen, no silver is dissolved, but when in an atmosphere of carbon dioxide, the water quickly acquires oligodynamic activity. Markvoort and Wieringa (22) found the same thing to be true. Under these conditions, it is possible that the silver changes to the oxide which is slightly soluble.

Krepelka and Toul (16) state that the maximum amount of silver which may be dissolved by water at 20° C. is 0.036 mg. per liter in twenty-one days. They also believe that the silver dissolves in the form of silver oxide, and they found that the solubility is slightly higher in glass vessels than in silver, probably due to the alkalies dissolved from the glass which aid in the solution of the silver.

Because of the time involved, this method of producing silver in solution would not lend itself readily to the commercial preservation of waters and beverages.

Development of Silvered Sands. Accordingly, a form of silver was developed by Prof. G. A. Krause, as reported by Salmony-Karsten (31), which will produce an oligodynamic effect quite rapidly. In this process, silver is blown on suitable carriers, with the addition of gold and palladium as catalysts, forming a fine coating of non-colloidal microcrystalline to sub-microscopic form which has a bactericidal action due to the giving up of silver to the solution. Water of low bacterial count may be sterilized in one minute to one hour, a higher count necessitating a longer exposure,

i. e., 21,000,000 bacteria per c.c. requiring forty-eight hours exposure to silvered sand.

This silver of Krause's is blown on sand, porcelain rings, or any other suitable medium which affords a large amount of surface for the silver. Silvered porcelain rings will give to water a silver concentration of 0.03 p.p.m. in one hour, 0.07 p.p.m. in two hours, 0.15 p.p.m. in three hours, and 0.5 p.p.m. in four hours according to the work of Gibbard (7).

Now the question arises as to what form this silver in solution takes. Is it metallic or is it ionic? To what is its toxicity due?

Toxicity Due to Ions. Wernicke and Modern (42), Egg and Jung (3), van Acker (39), Freundlich and Söllner (6), Hocs (9), Hoffman (11), Markvoort and Wieringa (22), and Leitner (19) state that the oligodynamic action of metals is due to and identical with that of metallic ions. Mathews (24) reports that some metals, silver included, are more active in the ionic than in the molecular state. These ions seem to hold their charges less firmly than others and hence are good oxidizers.

Vaindrach (38) reports that oligodynamy is regarded as a catalytic oxidative action caused by traces of metal adsorbed by the living organism.

Egg and Jung (3), after stating that the active principle in oligodynamy is the silver ion, point out that sligh-

tly dissociated silver salts, such as silver sulfide, have no sterilizing action. Colloidal solutions of silver and solutions containing complex silver ions may show oligodynamy, but this phenomenon is due to the liberation of silver ions through secondary reactions.

As early as 1897, Krönig and Paul (17) realized that the metallic ion possessed a germicidal activity and they reported that this activity in general was proportional to the degree of dissociation of the metallic compounds.

Höber (10) believes that penetrability of the ion into the cell is more important than dissociation. He found that mercuric nitrate had less toxic effect on anthrax spores than the halides though it was much more highly dissociated. This he associated with the fact that the nitrate is less soluble in lipoids than the halides. Schumaker (35) brings out the same fact in his studies of disinfection by dyes. The acid dyes are usually water soluble and innocuous, the basic dyes are lipoid soluble and toxic. These lipoid soluble substances combine with the karyoproteids of the nuclei of organisms. These karyoproteids contain lipoids. Could it not be possible that the silver ion might have a similar property of combining with the karyoproteids?

Critical Concentrations. Myers and Mauer (26) tested five forms of silver solutions in water sterilization. They tested solutions made from fused silver chloride, silver nitrate, colloidal silver produced by passing an arc be-

tween two silver electrodes immersed in distilled water, silver acetate, and electro-Katadyn silver water prepared by passing a small electric current of a few milli-amperes between two specially prepared Katadyn electrodes (Krause's silver metal). Their results obtained from these five types of silver solutions indicated that a concentration of from 0.10 p.p.m. to 0.15 p.p.m. of silver ions, irrespective of their source, was sufficient to destroy Escherichia coli, when present to the extent of 250,000 per c.c. within a period of two and a half hours. Staphylococcus aureus appeared to be only slightly more resistant to the action of the silver ion than Escherichia coli. The waters used for their experiments were sterilized river water having a slightly alkaline reaction and the same river water adjusted to the neutral point by the addition of ten parts per million of calcium carbonate.

Martinez (23) set up experiments using Bacillus typhi, Escherichia coli, Bacillus dysenteriae, Pneumococcus, Streptococcus, and Staphylococcus in concentrations of 1,000,000 per c.c. and subjected these water solutions to silver sand. Two hours (0.07 p.p.m. of silver) were necessary to kill typhoid, disentary, coli, pneumococci, and streptococci organisms, while three hours (0.15 p.p.m. of silver) were necessary for Staphylococcus.

Salmony-Karsten (32), (33) were able to kill Bacillus tuberculosis in forty-eight hours contact with act-

ivated silver (sand, etc.) when the organisms were present in concentrations of 20,000 per c.c.

A concentration of 0.04 milli-grams of silver per liter sterilized cultures of Escherichia coli in twenty-four hours, and a marked effect was noted by Egg and Jung (3) with concentrations as low as 0.001 milli-grams per liter.

A "standard silver water" containing 480 gammas of silver per liter (0.048 p.p.m.) will kill 5,000 colon bacili in two hours at 37° C. according to Eichbaum (4).

Viesohn (40) and Moiseev (25) report that the so-called water bacteria are less susceptible than Escherichia coli and that pathogens are more susceptible to silver ions.

Recent work by Just and Szniolis (13) indicate that in waters of slight contamination a silver concentration of 100 gammas per liter will sterilize the water in three to five hours. In open containers, 200 gammas per liter are necessary and a replacement of sixty to seventy gammas per liter daily is necessary to replace that which may be plated out on the container. Freundlich and Söllner (6) state that these silver ions adsorbed to the walls of the container may be given up again to the micro-flora and micro-fauna, hence a daily replacement is not absolutely necessary.

For culture yeast, 0.025 milli-grams of silver per liter were required for sterilization, compared with 0.1 milli-grams for wild yeast and 0.4 to 0.5 milli-grams for Sarcina, according to Lüers (21).

The above references indicate that micro-flora and micro-fauna in solution may be killed by the addition of minute quantities of silver. They also show that the effect is due to the positively charged silver ion. But just what actually takes place in solution? What effect does the ion have on the organism? Is the organism actually killed or is it merely inactivated in some way similar to the action of freezing temperatures on organisms? The following references indicate that there is a difference of opinion on these questions amongst the various workers in this field.

Characteristics of Germicidal Action. Traube (37) reports that metal ions are classed amongst those disinfectants which produce irreversible coagulations or denaturation and hence kills the organisms outright.

According to Schumaker (34), the ions of heavy metals penetrate the cell nuclei and form salts with the nucleic acids of the bacterial nuclei, producing death of the cell.

Leitner (18), and Liese and Mendel (20) are inclined to believe in the inhibition of growth theory. Liese and Mendel (20) report that the silver ion from a silver nitrate solution of definite concentration is primarily adsorbed to the cell surface, leading to an inhibition of growth without necessarily killing the cells. This latter occurs only when the silver is demonstratable within the cell by the leucomethylene blue method of Schumaker (36).

Leitner (18) noted that electrolytes hinder this bac-

tericidal effect of copper and silver salts. He reports that this inhibition is of neither a chemical nor a physico-chemical origin due to any action exerted by these added electrolytes on the metal ions. The electrolyte does not alter the concentration of these metal ions in solution, but the inhibition seems to be associated with a hindrance of the adsorption of the metal ions by the bacteria. Since the adsorption of electro-positive ions on bacteria would be greater where the bacteria carry a greater negative charge, then this inhibition of the germicidal effect of copper and silver salts by the addition of electrolytes is the result of a lowering of the negative charge on the bacteria. On this basis, increased alkalinity should increase the negative charge on the bacteria and hence increase the bactericidal effect of copper and silver, a fact easily verified experimentally.

Now that we have some idea regarding what happens to the bacterial cell when subjected to silver ions, what effect would these same silver ions have on the human body if consumed with the food and drink?

Ionic Silver and Higher Organisms. Just and Szniolis (13) report that higher organisms, such as fish and crustaceans, e.g., Daphnia magna, suffer visibly in a short time when living in water carrying a concentration of fifty gammas of silver per liter. But rats red with water containing from 400 to 1000 gammas of silver per liter did not show any inhibited development.

Neergaard (27),(28) and (29), in his studies of intravenous silver therapy, states that inorganic ions other than the chlorine ions found normally in physiological solutions have no material effect upon the concentration of silver ions which can be reached in the body. The highest possible concentration of silver ions is about 0.0001 milligrams per liter. When dissociated silver is added, the silver ion concentration in the blood increases far more slowly than in an isionic electrolyte solution, and it requires a some five-hundred fold amount to attain the maximum concentration, so that an amount of about 750 milli-grams per liter is necessary to attain this end. The component of blood which causes it to differ so greatly from a pure electrolyte solution is the albumin. The formation of compounds with chlorine and with albumin occur simultaneously, and there is an equalibrium between the two reactions. It is these reactions which inhibit the formation of silver ions in the human body.

According to Neiser and Eichbaum (30), a human being would consume only fifty milli-grams of silver during the course of a year if all his drinking water were sterilized with silver (0.015 g./ cu.m.). This quantity is far below the dosages which have frequently been used in intravenous silver treatment for therapeutic purposes. They state that some authors have administered as much as four and one-half grams of silver without harmful consequences. Another author

reports that fifteen to thirty grams of silver are necessary for producing argyria. Furthermore, the use of silver table ware and drinking ware for centuries, and the replacement of pieces of bone with silver plates without injury to health refutes any contention that there is any danger by such small quantities of silver that are contained in oligodynamically sterilized drinking water and beverages.

PROCEDURE

There are three general methods of producing silver ions in solution; the static-Katadyn method of using silvered sand, porcelain rings, etc., the electro-Katadyn method of using two Katadyn silver electrodes and a small electric current, and the method of deriving the ions from some silver salt in solution. According to Krause (14), Gutschmidt (8), and Viesohn (40), the electro-Katadyn method is far superior to the static-Katadyn method. Therefore, in the following investigations, the electro-Katadyn method and the salt solution method were used in producing silver ions in solution.

Samples of the various fruit juices studied, clarified and unclarified, of known yeast count, were given different concentrations of silver by the two methods mentioned above. Some of these treated samples were incubated and some were left at room temperature. All were held in glass containers. Signs of fermentation, mold growth, taste changes,

and color changes were noted.

Soluble solids determinations were made with a Zeis Refractometer at 20° C.

Sugar and acid determinations were made according to the Official Methods of the A.O.A.C., (1).

The hydrogen ion concentration was determined with a Leeds and Northrup quinhydrone electrode.

Silver determinations were made according to the author's modification of the method used by Jendrassik and Papp (12) in wine analysis.

The apple juice was clarified by filtering in a vacuum filter with the aid of 1% of Hy-Flo Super-Cel obtained from the California and Hawaiian Sugar Refining Corporation, San Francisco, California. The pear and loganberry juices were clarified with the use of Pectinol "W", an enzymatic preparation produced by Röhm and Haas Company, Philadelphia, Pennsylvania. This was used at the rate of four and one-half grams per gallon.

Where the count was large, micro-biological counts were made by the direct microscopic count method with the use of a Howard counting chamber. Where the count was small, the determinations were made by the dilution and plating-out method as employed in milk and water analyses.

The Saccharomyces ellipsoidaus culture used was obtained from the Bacteriology Department of O. S. A. C.

INVESTIGATIONS

Apple Juice. Ten of eleven samples of 250 c.c. volume of freshly pressed, unclarified apple juice testing 14.0% soluble solids, with a pH of 4.0, and a yeast count of less than 100,000 per c.c., were treated in 300 c.c. Erlenmeyer flasks with the electro-Katadyn activator for various lengths of time using two milli-amperes of current. The eleventh sample was untreated and was kept for a check. The resulting silver concentrations were calculated from the following formula:

$$\text{Conc. Ag. (gammas / liter)} = \frac{\text{milli-amps. x gammas / milli-amp.-hr.}}{\text{flow (liters / hr.)}}$$

The flow in liters per hour is found by multiplying the cubic centimeters treated by 0.06 and dividing this by the time of treatment. For example, 250 c.c. treated for three minutes would equal 250 x 0.06/3 or 5 liters per hour.

The number of gammas of silver released per milli-ampere-hour depends upon the current efficiency of the particular juice. From Faraday's Law (2), we find that one milli-ampere-hour of current would theoretically release 4.025 milli-grams of silver from the electrodes if the current efficiency of the system were 100%. But in the case of this apple juice, the current efficiency was found to be only 15%, hence, only 0.604 milli-grams (604 gammas) of silver were released by one milli-ampere-hour. Therefore, knowing the vol-

ume treated, the time and amperage of the treatment, and the current efficiency of the system, the resulting silver concentrations may be calculated quite readily. For this experiment, they were as follows:

TABLE 1

Activation Degree of Unclarified Apple Juice No. 1

(100,000 yeast per c.c.)

Time at 2.0 m. a. (in min.)	Silver Content (gammas / liter)
0	0
2	161.0
4	322.1
6	483.2
8	644.2
10	805.0
20	1610.0
25	2013.0
30	2416.0
35	2815.0
40	3221.0

These samples were plugged with cotton after treatment, placed in the incubator at 83° to 86° F., and examined periodically for yeast and mold growth.

The untreated sample was found to be fermenting after one day of incubation, while none of the treated samples fermented even in thirty-three days of incubation.

Mold growth developed in the untreated sample in four days, and in the treated samples in inverse proportion to the silver content of the samples as shown by the following

table:

TABLE 2

Mold in Treated Apple Juice No. 1

(100,000 yeast per c.c.)

Silver Content (gammas / liter)	Time of Appearance (in days)
0	4
161.0	5
322.1	5
483.2	9
644.2	9
805.0	13
1610.0	17
2013.0	27
2416.0	None in 33
2815.0	None in 33
3221.0	33

Another set of seven 250 c.c. samples of the same juice but with a yeast count of 150,000 per c.c. was given varying amounts of silver with the electro-Katadyn activator according to this schedule:

TABLE 3

Activation Degree of Unclarified Apple Juice No. 2

(150,000 yeast per c.c.)

Time at 2.0 m.a. (in min.)	Silver Content (gammas / liter)
0	0
4	322.1
8	644.2
10	805.0
20	1610.0
30	2416.0
40	3221.0

After treatment, these samples were transferred to 250 c.c. prescription bottles, tightly capped, and kept in a dark room at 68° F. They were examined periodically for yeast and mold growth.

No mold growth appeared in any of the samples even after ninety days storage.

Fermentation began in the untreated sample within three days and in the others in inverse proportion to their silver contents, as shown by the following table:

TABLE 4

Fermentation of Treated Apple Juice No. 2

(150,000 yeast per c.c.)

Silver Content (gammas / liter)	Time of Appearance (in days)
0	3
322.1	10
644.2	26
805.0	44
1610.0	None in 90
2416.0	None in 90
3221.0	None in 90

Another experiment was conducted using clarified apple juice testing 10.8% soluble solids and 6.8% total sugars, and having a pH of 4.0 and a yeast count of about 15,000 per c.c. Nine samples of 250 c.c. volume were treated with the electro-Katadyn activator for various lengths of time using three milli-amperes of current. The activation times

and the resulting silver contents were:

TABLE 5

Activation Degree of Clarified Apple Juice No. 1

(15,000 yeast per c.c.)

Time at 3.0 m.a. (in min. and sec.)	Silver Content (gammas / liter)
0	0
40 sec.	80.5
1 min. 20 sec.	161.0
2 min.	241.6
2 min. 40 sec.	322.1
5 min.	604.0
8 min.	966.4
12 min.	1449.6
16 min.	1932.8

After treating, the samples were placed in 250 c.c. prescription bottles, tightly capped, and the Tyndall phenomenon noted. This was found to be greatest in those samples containing the most silver.

These samples were left in a dark room at 68° F. and observed periodically for yeast and mold growth.

The non-silvered sample began to ferment in two days, but the silvered samples had not begun to ferment even after seventy-seven days.

After five days storage, all of the treated samples but that one given 1932.8 gammas of silver per liter had developed a white anaerobic mold growth on the bottom of the container, the number of colonies being greatest in those samples containing the smallest amount of silver. By the

end of eight days storage, this sixteen-minute treated sample also had developed two of these white fluffy colonies of mold. The mold colonies in all the samples grew in size for about a week and then apparently died.

Pear Juice. Ten 250 c.c. samples of unclarified Bosc pear juice testing 15.4% soluble solids and having a pH of 4.25 were given inoculations of a culture of Saccharomyces ellipsoideus to give various yeast counts, as follows:

TABLE 6

Yeast Count of Unclarified Bosc Pear Juice No. 1

No. of 250 c.c. Samples	Cells per c.c.
2	147,593
2	216,824
2	286,054
2	562,967
2	839,901

One sample of each yeast count was activated with the electro-Katadyn set for twelve minutes using two milliamperes of current, while the duplicates of these were not silvered. The current efficiency of pear juice was found to be about 20%, consequently, these treated samples contained 1,288 gammas of silver per liter. These ten samples in cotton-plugged 300 c.c. Erlenmeyer flasks were incubated at 86° F. and observed periodically for fermentation.

After one day of incubation, all of the non-silvered samples were fermenting, those of highest original yeast

count were fermenting most rapidly. Of the treated samples, the two of the highest yeast count began to ferment in four days and those of the other three yeast counts began in five days.

Another series of five samples of the same Bosc pear juice was given a heavier inoculation of yeast from the culture. After inoculation, these samples were given a silver content of 3,599 gammas per liter by the use of the electro-Katadyn set and incubated at 86° F., and observed periodically for any signs of fermentation. The yeast contents and the number of days before fermentation appeared, were as follows:

TABLE 7

Unclarified Bosc Pear Juice No. 2

Code	Yeast Count per c.c.	Fermentation Appearance in Days
T7	1,348,793	None in 83
T10	1,863,593	None in 83
T15	2,721,593	None in 83
T20	3,579,593	19
T25	4,437,593	10

In another experiment, freshly pressed, unclarified D'Anjou pear juice with a small micro-flora count of about 50,000 per c.c., was given a treatment with the electro-Katadyn set. The original juice tested 13.2% soluble solids, 10.16% total sugars, 9.12% reducing sugars, and pH 4.25.

Five samples of 250 c.c. volume were treated for various lengths of time. After treatment, these cotton-plugged Erlenmeyer flasks were placed in the incubator at 86° F. and examined periodically for yeast and mold growth.

The non-silvered sample was discarded before any mold growth appeared, but fermentation and mold growth appeared in the others as follows:

TABLE 8

Unclarified D'Anjou Pear Juice
No. 1

(50,000 cells per c.c.)

Silver Content (gammas / liter)	Fermentation Appearance (in days)	Mold Growth Appearance (in days)
0	2	-
1333	7	10
2000	None in 49	37
2666	None in 49	None in 49
3333	None in 49	None in 49
4000	None in 49	None in 49

Six more samples of this same freshly pressed D'Anjou pear juice were inoculated with a yeast culture to give different yeast counts. After inoculation, they were given a silver content of 2,666 gammas per liter with the electro-Katadyn set, the flasks plugged with cotton, and incubated at 86° F., and examined periodically for yeast and mold growth.

No fermentation occurred in any of the samples in

forty-nine days, but mold growth appeared as follows:

TABLE 9

Unclarified D'Anjou Pear Juice
No. 2

(2,666 gammas Ag. per liter)

Yeast Count (cells per c.c.)	Mold Appearance (in days)
500,000	None in 49
1,000,000	None in 49
1,500,000	23
2,000,000	28
2,500,000	23
3,000,000	24

In another experiment with D'Anjou pear juice, silver ions derived from silver salts were used. Acqueous stock solutions of silver nitrate, silver sulphate, and silver lactate were made up so that 1 c.c. yielded 250 gammas of silver. At this small concentration, it is believed that all the silver salt is in the ionized form (or "active" form, according to the new 100% ionization theory).

Proper amounts of these solutions were mixed with D'Anjou pear juice to give in the 250 c.c. samples a silver content ranging from 200 gammas per liter to 3,000 gammas per liter. The juice used was the unclarified juice employed in the previous experiment, but with a contamination count of about 1,000,000 per c.c. After treatment, these samples in loosely capped prescription bottles were incubated at 90° F. and periodically examined for yeast and mold growth.

The following table indicates the manner in which fermentation and mold growth appeared in the samples:

TABLE 10

Unclarified D'Anjou Pear Juice No. 3

(treated with silver salts)

Ag. Content Gammas per liter	Appearance of Fermentation - in Days			
	$AgNO_3$ added	Ag_2SO_4 added	$AgC_3H_5O_3$ added	Mold Appearance
200	2	2	2	5
400	5	4	4	5
600	4	8	8	5
800	6	None in 62	None in 62	5
1000	None in 62	None in 62	None in 62	5
2000	None in 62	None in 62	None in 62	11
3000	None in 62	None in 62	None in 62	22

Another experiment was conducted using the same unclarified D'Anjou pear juice as above. One sample was given an inoculation from the silver nitrate stock solution, giving it a silver content of 2,400 gammas per liter, and three other samples were treated with the electro-Katadyn activator giving silver contents of 1,333, 2,666, and 3,999 gammas per liter to the samples. All four samples were in cotton-plugged Erlenmeyer flasks and were incubated at 86° F.

In one day, all of the Katadyn treated samples turned a greyish-green color as viewed by reflected light, the sample with the most silver being darkest in hue. The silver nitrate sample did not develop this color. In sixteen days, the sample with 1,333 gammas of silver per liter had almost totally cleared, throwing down a sediment, while the other

Katadyn treated samples had partially cleared, throwing down a lesser amount of sediment.

None of the samples fermented in sixty-two days, but the silver nitrate treated samples had developed an anaerobic mold growth.

Loganberry Juice. Heat pasteurized loganberry juice of pH 3.06 and 11% soluble solids was clarified with Pectinol "W", the clarified juice being re-pasteurized by heating to 180° F. After cooling, it was given a yeast count of 500,000 per c.c. by the addition of the proper amount of yeast culture. This clarified juice had a pH of 3.03 and a soluble solids content of 10.6%. Using the three stock silver salt solutions, samples of this loganberry juice in prescription bottles were given silver contents ranging from 200 to 3,000 gammas per liter, placed in the incubator at 80° F., and observed for fermentation.

All samples were fermenting at the end of one day of incubation.

Another series of samples were given silver contents ranging from 1,000 to 6,000 gammas per liter by the addition of the proper amounts of the stock solutions. These also were incubated at 80° F., and observed for fermentation.

After one day of incubation, all of these samples were fermenting.

Another series was set up in which the silver contents ranged from 7,000 to 15,000 gammas per liter.

All of these also were fermenting after one day of incubation.

An experiment, then, was set up in which the pH of the juice was adjusted to 5.0 by the addition of sodium hydroxide solution. The yeast count of this adjusted juice was built up to 500,000 per c.c. by the addition of the proper amount of culture and given the following silver contents, using the silver sulphate solution; 2,000, 7,000, and 15,000 gammas per liter, respectively. These samples were incubated at 80° F. in loosely capped prescription bottles.

Table 11 indicates the number of days of incubation before fermentation appeared in these samples:

TABLE 11

Clarified Loganberry Juice of pH 5.0

(500,000 yeasts per c.c.)

Silver Content (gammas / liter)	Fermentation Appearance (in days)
0	1
2000	3
7000	10
15000	18

The pH of all the samples had dropped to a pH of 4.1 to 4.5 at the time that fermentation began.

Another experiment was set up in which the pH of pectinol clarified loganberry juice was changed by adding

different amounts of sodium hydroxide solution to give different pH values as follows: pH 3.1, pH 3.59, pH 4.08, pH 4.52, pH 4.96, pH 5.58, pH 6.13 and pH 6.69. The soluble solids contents of all these samples were 10.2%. The yeast count was increased to about 400,000 per c.c. by adding the proper amount of culture. The juice of each pH value then was divided into three samples of 200 c.c. volume, one given 1,500, one 2,500, and one 3,500 gammas of silver per liter by the addition of silver sulphate solution. These samples in loosely capped prescription bottles were incubated at 80° F., and observed periodically for fermentation. The results were as follows:

TABLE 12

Loganberry Juice with Adjusted pH Values

(treated with Ag_2SO_4)

pH Values	Hours for Fermentation to Appear		
	1500 gammas Ag. per l.	2500 gammas Ag. per l.	3500 gammas Ag. per l.
3.1	17	17	17
3.59	17	17	17
4.08	17	17	17
4.52	17	17	22
4.96	17	22	65
5.58	41	88	88
6.13	41	136	160
6.69	88	184	208

Action of Silver on Yeast. A loop of yeast from the bottom of a sample of silver treated Bosc pear juice which had not begun to ferment after twenty-two days incubation at

86° F., was diluted with about 150 c.c. of sterile pear juice. This dilution was incubated for one day without any fermentation taking place and then a portion was streaked out on wort agar and incubated. After three days of incubation at 86° F., a growth appeared on the agar which by microscopic examination was found to be a pure culture of yeast (Saccharomyces ellipsoideus, probably).

After forty-nine days of incubation, the original treated Bosc pear juice had not begun to ferment, so the same determination as above was made. After incubating the streak for three days, no growth appeared on the agar. All the yeast was apparently dead.

DISCUSSION OF RESULTS

It appears from the above experiments with apple and pear juice that yeast growth may be stopped by the action of silver ions, but that mold growth can not be stopped by silver ions alone. With the apple juice, mold growth was checked only when the bottles were capped tightly, thus excluding air and decreasing to a minimum the oxygen available for growth. But even when this precaution is taken, an anaerobic mold growth may appear.

From the apple juice experiments, it may be concluded also that this preservative action of silver is more effective at 86° F. than at 68° F. This point was evidenced by the fact that the unclarified juice with about 100,000 yeasts

per c.c. did not ferment when held at 86° F. even with the sample containing 161 gammas of silver per liter, but the same juice when held at 68° F., exhibited fermentation even in the sample containing 805 gammas of silver per liter. This fact was reported also by Gibbard (7) and by Markvoort and Wieringa (22). Gibbard (7) found that temperatures of 8° to 10° C. as compared with 22° to 25° C. markedly retarded the oligodynamic action of silver. With the higher temperatures, more killing was observed. Markvoort and Wieringa (22) report that the dying off of bacteria is greatest at 28° C.

It also was noted from these apple juice treatments that it required less silver to keep the clarified juice than the unclarified juice. All of the silvered clarified apple juice samples kept when held at 68° F., while only the three of the unclarified samples which had the greatest silver contents kept more than forty-four days when held at the same temperature. This is undoubtedly due to the fact that in the unclarified juices there is a large amount of suspended material to which much of the silver is adsorbed, thus leaving a lesser amount available to act upon the micro-organisms.

Other things being equal, the yeast count is an important factor in the preservation of juices with silver. It was noted that with the Bosc pear juice, a silver content of 1,288 gammas of silver per liter retarded the yeast growth for four days when yeasts were present to the extent of

839,901 per c.c., but this same amount of silver retarded the growth for five days when the yeast count was 286,054 per c.c. When about 3,600 gammas of silver per liter were present, no fermentation occurred in eighty-three days, even when the yeast count was roughly 2,720,000 per c.c., but fermentation took place in nineteen days when 3,600,000 per c.c. were present, and in ten days when 4,440,000 per c.c. were present. From this, it appears that there must be a definite minimum silver ion concentration for each yeast concentration in order to stop the growth of the yeast cells. When 2,700,000 yeast cells per c.c. are inhibited from growing by a silver ion concentration of 3,000 gammas per liter, the author has calculated that the silver ion concentration is 18.5×10^{11} for each yeast cell. Not all of these ions come in contact with the cell however, but this number must be present in order that enough ions will come in contact with the cell to inhibit its growth. In the treatment of drinking water where 100,000 Escherichia coli organisms per c.c. were present, Fresenius (5) reports that 24×10^{11} silver ions per bacterium must be present to kill the cell. The number here is smaller than above because of the difference in acidities of the two media and because of the different organisms involved.

With ordinary contaminations in freshly prepared pear juice, about 50,000 per c.c., a silver content of 2,000 gammas per liter will keep the juice from spoiling for at least forty-nine days, the length of this experiment, and the author

believes that this amount of silver will inhibit the yeast growth for a much longer time, for 2,666 gammas per liter kept for forty-nine days juice which had 3,000,000 yeast cells per c.c.

It was noted in this last experiment with pear juice employing 2,666 gammas of silver per liter, that mold growth occurred in inverse proportions to the yeast count of the juice, that is, with the samples of high yeast count, mold appeared first, and with those of 1,000,000 yeasts per c.c., no mold appeared. Could not this be due to the fact that with the high yeast counts the silver ions are withdrawn more rapidly from the surface film, thus leaving an ideal place for the mold spores present to produce growth?

With pear juice as with apple juice, it was brought out that it is much easier to keep clarified juice than unclarified juice. With the clarified juice, 1,000 gammas of silver per liter preserved the juice even with a count of 1,000,000 yeast cells per c.c., while with the unclarified juice, a concentration of 2,000 gammas per liter was necessary.

These experiments with pear juice also indicated that silver ions derived from silver salts in solution, were just as effective as the silver produced by the electro-Katadyn method. Myers and Mauer (26) and Markvoort and Wieringa (22) obtained the same results in their work on water sterilization.

There is a slight color change in these light colored pear and apple juices which is found only in the Katadyn treated samples and not in those treated with silver salts. This opalescent green color noted and the Tyndall phenomenon observed, indicates that much of the Katadyn silver produced is either colloidal in state or produces a reaction within the juice which increases the colloidal content of the juice. Kreipe (15) noted the same change in appearance when treating vinegars by the Katadyn method. He reports that an opalescent turbidity was noted in concentrations of 850 gammas of silver per liter and above. The author observed that this color change also was more pronounced at the higher temperatures of storage.

With the silver treated pear and apple juices, a silver concentration of 1,000 gammas per liter gave a slight metallic taste which was more pronounced when the juice was held at 86° F. than at 68° F. This taste increased with greater concentrations of silver. It was noted in the Katadyn treated samples as well as in the silver salt treated samples.

That the reaction of the fruit juice or beverage to be sterilized by the ionic silver method is a very important factor was exhibited very clearly with the loganberry juice experiments. With juice of pH 3.03, it was impossible to preserve it even with a silver content of 15,000 gammas of silver per liter (15 p.p.m.). It was shown that if the re-

action of this juice was adjusted to a lower acidity, the juice could be preserved for as long as 208 hours with a silver ion concentration of 3,500 gammas per liter. But with the method employed, the pH tended to return to its original value thus allowing fermentation to proceed. This pH or reaction factor is very interesting, and the author believes that it is explained somewhat by Leitner (19), as discussed in the early part of this paper. It is supposed that the positively charged silver ion is attracted to and adsorbed on the microorganism. A highly negative charge on a bacterium or yeast would more readily attract and adsorb the silver ion than a lesser negative charge. It can be shown that a high pH lowers the negative charges on such bodies and conversely that a low pH raises the intensity of the negative charge. Therefore, an organism in a highly acid medium would carry a lower negative charge than when in a less acid medium, and hence would not be killed as readily by the silver ion. This explains fairly well one concept of the effect of reaction in silver sterilization.

Looking at the phenomenon from another angle, it might be said that with a lower pH, the positive ion concentration of the system is greatly increased thus producing a greater competition for adsorption between these positive ions, resulting in a preferential adsorption of the lighter and less dense hydrogen ions over the heavier silver ions. The hydrogen ion might be spoken of as being more "motile"

than the heavy silver ion, if one may speak of motility of ions.

Whatever the explanation of this factor may be, it still remains that the pH of the solution is equally important as the kinds and numbers of organisms present in the juice to be treated.

The experiments with silvered pear juice, in which an attempt was made to find out just what happens to the yeast cell when it is subjected to the treatment with silver ions, indicated that not all of the cells are killed instantly, as reported by Traube (37) and Schumaker (34), but are only inhibited from growing. This corresponds to the viewpoint of Leitner (19) and Leise and Mendel (20). But the experiments also showed that eventually the yeast cells die. From outward appearances this phenomenon is much like that which occurs in frozen pack fruits held at a temperature of about 0° F. Growth of the organisms are inhibited by the low temperatures of storage and during storage their numbers decrease steadily. This is thought to be a dehydrating process in which the organism is eventually killed. Could not this reaction of the silver ions on the organisms also be a dehydration process? Traube (37) indicates that such is the case.

SUMMARY

1. Yeast growth in pear and apple juice may be stopped with a silver content of 2,000 gammas per liter in unclarified juices of nominal contaminations, and with 1,000 gammas per liter in clarified juices.

2. The silver ion is the preserving factor in silver preservation, and is equally potent regardless of its source, i.e., electro-Katadyn or from silver salts.

3. Mold growth can be stopped only by some method of excluding air from the headspace of the container in addition to silver treatment.

4. The efficiency of the silver ion is higher at 86° F. than at 68° F.

5. Katadyn silver produces an opalescent hue in the juices while silver from salt solutions does not. This color change is greater at increased temperatures.

6. A slight metallic flavor is given to the pear and apple juices treated with silver salts and Katadyn silver. This taste is noted with silver concentrations as low as 1,000 gammas per liter.

7. The acidity of the juice is just as important as the kinds and numbers of organisms present.

8. Because of the low pH, loganberry juice at present does not adapt itself to preservation with silver.

9. Micro-organisms are first inhibited from growing,

but are finally killed after some length of time of exposure to silver ions.

LITERATURE CITED

(1) Association of Official Agricultural Chemists. Official and tentative methods of analysis, third edition. Washington, D. C., 1930.

(2) Chemical Engineers' Handbook. McGraw-Hill Book Co., New York, first edition, pp. 2298-2304, 1934.

(3) Egg, C. and Jung, A. Mikrochem. Pregl Festschr., 46-60, 1929.

(4) Eichbaum, Franz. Zentr. Bakt. Parasitenk I Abt. 126, 128-134, 1929.

(5) Fresenius, L. Deutsche Baeder u. Brunnen Zeitung, 18, Sept. 1932.

(6) Freundlich, H. and Söllner, K. Biochem. Zeitung, 203, 1-13, 1928.

(7) Gibbard, J. Can. Pub. Health Jour. 24, 96-7, 1933.

(8) Gutschmidt, H. Z. Hyg. Infektionskrank 116, 421-32, 1934.

(9) Hocs, S. Helv. Chim. Acta 13, 153-72, 1930.

(10) Höber, R. Physikalische Chemie der Zelle und der Gewebe, Ed. 5, 1924.

(11) Hoffman, Paul. Zentr. Bakt. Parasitenk I Abt. 114, 216-27, 1929.

(12) Jendrassik, A. and Papp, Sz. Silberbestimmung in Katadynisierten Weinen und sonstigen flüssigen Lebensmitteln. Zeitschrift für Untersuchung der Lebensmittel, heft 4. band 69, April, 1935.

(13) Just, J. and Szniolis, A. Gaz. i. Woda 15, 201-11, 1935.

(14) Krause, Geo. A. Wasser 7, 74-89, 1933.

(15) Kreipe, Heinrich. Die Deutsche Essigindustrie 37, 32, 257-259, 1933.

(16) Krepelka, H. and Toul, F. Collection Czechoslov. Chem. Comm. 1, 156-64, 1929.

(17) Krönig, B. and Paul, T. Ztschr. Hyg. u. Infektionskrank, 25, 1-112, 1897.

(18) Leitner, N. Klin. Wochschr. 8, 1952-7, 1929.

(19) _____ Biochem. Z., 221, 43-63, 1930.

(20) Liese, W. and Mendel, B. Ztschr. Hyg. u. Infektionskrank, 100, 454-471, 1923.

(21) Lüers, H. Wochschr. Brau. 49, 377-81, 1932.

(22) Markvoort, A. J. and Wieringa, K. T. Über oligodynamische Wirkung von Metallen. Chem. Weekbl. 29, 242-247, 1932.

(23) Martinez, Don Edwardo Gallardo. Certificado No. 298, Instituto Nacional de Higiene de Alfonso XIII., Madrid, Sept. 20, 1929.

(24) Mathews, A. P. Physiological Chemistry, 5th ed. p. 237, W. Wood and Co., N. Y., 1930.

(25) Moiseev, S. V. J. Am. Water Works Assoc. 26, 217-38, 1934.

(26) Myers, Robt. P. and Mauer, J. C. J. of Bact. 29, 1, pp. 7-8, 1935.

(27) Neergaard, K. van. II The effect of the electrolytes of the body on the solubility of silver salts. Arch. exptl. Path. Pharm. 107, 316-48, 1925.

(28) _____ III Solubility of silver salts in the blood and body fluids with reference to silver-protein combinations. Arch. exptl. Path. Pharm. 108, 295-312, 1925.

(29) _____ IV. The silver-ion concentration in the process of disinfection in physiological media. Arch. exptl. Path Pharm. 109, 143-63, 1925.

(30) Neiser, M. and Eichbaum, F. Ergeb. Hyg. Bakt. u. s. w. v. Wolfgang Weichhardt, 13, 170-226, 1932.

(31) Salmony-Karsten, A. Wasser u. Gas 20, 299-300, 1929.

(32) _____ Chem. tech. Rundschau 46, 551-3, 1931.

(33) _____ Wasser u. Abwasser 30, 274-5, 1932.

(34) Schumaker, Josef. Med. Klin., 18, 159-160, 1922.

(35) _____ Centbl. Bakt., Abt. 1, 67-80, 1926.

(36) _____ Chem. Zelle Gewebe 12, 433-72, 1926.

(37) Traube, J. Ztschr. Immunitäts, Teil. 1, Orig. 29, 286 -302, 1920.

(38) Vaindrach, G. M. Zhurnal. exptl. Biol. Med. 10, 206-11, 1928.

(39) van Acker, Alb. Natuurw. Tijdschr. 15, 93-104, 1933.

(40) Viesohn, J. Gesundh. Ing. 56, 316-19, 1933.

(41) Wernicke, R., Dortzenbach, R., and de la Barrera, J. Rev. inst. bacterial. dept. nacl. hig. 4, 375-84, 1926.

(42) _____ , and Modern, Ferdinand. Biochem. Zeitung, 214, 187-97, 1929.