THE UNIVERSITY OF MANITOBA

CHEMICAL PRESERVATION OF FRESH WHITEFISH FILLETS

By

WARREN GUNN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF FOOD SCIENCE

WINNIPEG, MANITOBA

OCTOBER 1975

"CHEMICAL PRESERVATION OF FRESH WHITEFISH FILLETS"

by

WARREN GUNN

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

The writer would like to express his appreciation to Dr. R.R. Pereira, Department of Food Science, for his guidance, suggestions, and encouragement in this study.

Sincere thanks are also extended to Dr. R.A. Gallop, Head of the Department of Food Science for guidance and support of this project, and to Marion Vaisey of Foods and Nutrition, Department of Home Economics for her valued advice and assistance.

I wish to express my appreciation for the assistance and advice given to me by professors and students of the Food Science Department, especially those who so generously donated their time to take part in the taste panels.

Special thanks finally go to Lori Gutkin who unselfishly donated her time to type the thesis manuscript.

ABSTRACT

The effect of Potassium sorbate, Disodium EDTA, and combinations of these salts on the Microbiological, Chemical, and Sensory qualities of fresh Whitefish fillets were studied.

The microbiological examinations on the treated fillet pieces indicated that the potassium sorbate treatments had significant inhibitory effects on the growth of spoilage bacteria. It was indicated that the growth and replication of Pseudomonas spp., the main spoilage organism was significantly inhibited by a concentration of 4% w/v for an exposure time of five minutes. Killing effects were observed as the concentration was increased to approximately 6 to 8% w/v for five minutes exposure time. Residual sorbate concentration was under the safe maximum permitted level only at the 8 to 10% w/v level. Therefore 6% w/v solutions could be used safely and effectively at a storage temperature of $3^{o}C$. Shelf life extensions of approximately fifteen days were observed at the 6% treatment level.

Organoleptic profiles of the cooked fish piece samples treated with potassium sorbate revealed effective shelf life extensions of from ten to fourteen days over controls. At the 8% level the presence of the chemical could be detected in taste and aroma after ten days storage at $3^{o}C$, but at a level of 6% potassium sorbate it could not be detected. Therefore a useful limit of 6% w/v potassium sorbate

for five minutes exposure time was determined to be approximately the maximum for best results for fourteen to twenty days storage at 3°C.

The calcium disodium salt of EDTA was found to be ineffective in preventing spoilage at the 4% level for five minutes exposure time, and in some cases the treatment was worse than controls after six days storage at 3°C. However, the disodium salt of EDTA was found to be effective in forestalling spoilage at the 6 to 8% levels for five minutes exposure time. Shelf life extensions of up to nineteen days over controls, from a bacteriological standpoint, were observed. However, since levels of 8 to 10% could be detected after ten days storage, a maximum useful treatment level would be from 4 to 6% w/v for five minutes exposure time. Residual levels of disodium EDTA in the fillet pieces did not exceed 700 p.p.m. for the 6% treatment level. Organoleptic evaluations revealed shelf life extensions of up to fourteen days for treatments as mild as 6% disodium EDTA.

When fish fillet piece samples were treated by a combination of potassium sorbate and disodium EDTA solutions at levels of from 4 to 6% w/v for a five minutes exposure time the best results were obtained from the standpoint of bacteriological shelf life extension, with shelf life extensions of from ten days for the 4% level to over twenty days for the 10% level. However, since taste panels revealed un-

desired effects on taste and aroma for levels over 8% w/v for five minutes exposure time, the treatment range of from 4 to 6% w/v for five minutes exposure time was found to be the best treatment. Both bacteriological and organoleptic factors were taken into account in arriving at this conclusion. When samples were treated with 6% solutions of the two chemicals one after the other, shelf life extension was determined organoleptically to be as much as fourteen days.

TABLE OF CONTENTS PAGE

PAGE

PAGE

PAGE

LIST OF APPENDIX TABLES

LIST OF APPENDIX TABLES CONTINUED

LIST OF APPENDIX TABLES CONTINUED

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES CONTINUED

LIST OF ABBREVIATIONS

FFMC	- Fresh Water Fish Marketing Corporation, Manitoba
EDTA	- Ethylenediaminetetraacetic acid
MeV	- million electron volts
Krad	- one thousand rad units of radiation
K-SA	- potassium sorbate
min	- minutes
sec	- seconds
log	- logarithm
APHA	- American Public Health Association
dist	- distilled
SPC	- Standard Plate Count or Total Plate Count
iu	- International units
mg	- milligrams
ml	- milliliters
gm	- grams
nm	- nanometers
mm	- millimeters
cm	- centimeters
oz	- ounce (Avoirdupois)
TBA	- thiobarbituric acid
U.S.A.	- United States of America
$L.D._{50}$	- Lethal dose for 50% of the population tested

INTRODUCTION

Of the total amount of freshwater fish marketed in the world today the major portion is consumed in the fresh unfrozen state, as compared with the frozen and processed states. This is dependent upon consumer preference and distribution systems. In lesser developed areas, due to lack of adequate refrigeration and transportation systems most of the seasonal catch is sold fresh in the local markets. The rest is cured or dried by traditional methods. In Canada, the United States, and Western Europe adequate refrigeration and reasonably good transportation facilities are available. There is a high demand for fish in more convenient forms.

In Manitoba approximately 60% of all production is marketed in the frozen whole or processed forms. Of the $ 13.28 million total sales of the Freshwater Fish Marketing Corporation (FFMC) in the period May 1/70 to April 30/71 (FFMC Annual Report, 1971), $4.59 million was sold as fillets. Of this amount Whitefish accounted for $1.35 million or approximately 30% of the total fillet sales. The last published preliminary totals for 1972 show increased sales volume to approximately $22.3 million (Canadian Fisheries Report, 1973). Whitefish is therefore important for its high price per pound and its high sales volume. It finds a ready market in Canada and the U.S.A. with 80% of all production

exported to the south.

Losses due to spoilage in the range of 10% (based on culls, production loss, and inventory loss) represent a loss of $ 460,000 per year to cooperatives such as the FFMC, with losses in Whitefish accounting for as much as $135,000. Lower prices per pound to the fisherman, and higher prices to the consumer, are the end results.

Indications are that fresh fillets exported to the U.S.A. undergo even greater amounts of spoilage with even entire carloads or truckloads going bad. (January 5/73 communication from R. Rastogi formerly of the FFMC). This spoilage is due in part to biochemical changes in the fish itself. However, microbiological aspects become very important as the raw product is further handled and processed and as the time from catch increases. Considerable savings would result if most of the spoilage could be eliminated in some way.

The freezing of fish fillets has largely overcome bacteriological spoilage. Fresh unfrozen fillets, however, seem to be more attractive to the consumer partly because of the 'fresh' appeal but more so because of the organoleptic superiority of fresh newly caught fillets over fresh frozen fillets.

Although the problems involved with keeping fillets fresh, begin at the time the fish is caught, they really start at the time they are sliced off the fish itself. In essence,

this procedure amounts to an inoculation of the fish muscle, normally sterile, with the normal psychrotrophic bacterial flora of the skin, slime, gills, and some of the intestinal fluid. If the temperature is not kept low (0-5^{o}C), growth and subsequent spoilage will occur rapidly.

Experience has shown that cool temperatures are insufficient to forestall fish spoilage, Castell et al.,(1949) ; Chai et al.,(1968); Evelyn and McDermott (1961); Lerke et al.,(1965); Levin (1968); Nair and Lahiry (1968); Shewan (1962) ; and Tama (1954). Maximum shelf life for clean Whitefish fillets at 1 to 3^{o}C is approximately 5 to 6 days. Although washing of the fish before scaling and filleting has been proven to significantly reduce the bacterial load, it requires large amounts of water. This extra usage would greatly increase the water pollution problems already existing at fish processing plants such as the FFMC. Therefore a chemical treatment for extending the useful shelf life of fresh fillets would be be preferred over a further washing treatment.

There is a continuing search for a suitable chemical which could be used to preserve fresh fillets, especially Whitefish fillets, and thus extend the normal shelf life at refrigeration temperatures (1 to 3^{o}C).

The problem involves the finding of a chemical or combination of chemicals, which could inhibit the oxidative spoilage reactions and bacterial growth, while being accept-

able from a public health standpoint. The highest effective concentration(s) have to be such that the residuals left in the fish are safe for human consumption with no long term side effects. Finally, if the treatment could meet all these criteria it would also have to be acceptable from an organoleptic point of view. That is, the chemical treatment should have no undersirable effects on the taste, smell, texture, or colour of the treated fish fillets.

The purpose of this study was to investigate the use of potassium sorbate and EDTA salts to preserve fresh Whitefish fillets. These two preservatives have been used for other fish products with significantly successful results. (See Literature Review pages 19-22 and 28-31 . An attempt was therefore made to achieve similarly successful results with fresh Whitefish fillets, which to date has not yet been done.

LITERATURE REVIEW

THE MICROBIAL FLORA OF FRESHWATER FISH ASSOCIATED WITH SPOILAGE

Flora Commonly Encountered

The deterioration of fish muscle due to bacterial growth overshadows all other causes of fish deterioration (Nair and Lahiry, 1968). The bacterial flora commonly associated with the spoilage of freshwater fish, in decreasing order of importance are the species from the following genera: Pseudomonas, Aeromonas, Micrococcus, and Lactobacillus, followed by: Escherichia, Brevibacterium, Paracolobactrum, Aerobacter, Sarcina, Proteus, Bacillus, Alcaligenes, Achromobacter, Streptococcus, Flavobacterium, and Chromobacterium (Evelyn and McDermott, 1961). Tarr (1954) reported that many of the spoilage bacteria are species of the genera Corynebacterium and Mycoplana.

Environmental Considerations

Shewan (1971) found that the above mentioned genera represent the microbial environment from which the fish were taken and that this phenomenon generally applied to any environment. He also found that the microbial pattern is dependant upon seasonal temperature, geographical location, species of fish involved, fishing method used, and the handling of the fish after the catch. Furthermore, variations in the microbial population pattern result from the changing

of any one or all five of these factors. Shewan (1962) showed that over 95% of the microorganisms in Northern waters are psychrotrophic. Water temperature then plays a major role in determining fish microflora.

Distribution of the Microflora

Nair and Lahiry (1968) found that the body fluids and muscle tissue of freshly caught fish were generally sterile. However, the slime, gills, and intestines carry high bacterial loads. Usually about 10^2 to 10^5 organisms per square centimeter are found on the skin or gills, while 10^3 to 10^5 organisms per ml are found in the intestinal fluid.

The Microbial Flora Of Cold Fresh Water Northern Lake Fish

According to Nair and Lahiry (1968), Pseudomonas, Achromobacter, Flavobacterium, Micrococcus, and Lactobacillus usually predominate on cold fresh water northern lake fish, while Sarcina, Proteus, Bacillus, and a few other minor genera are found in lesser numbers. At the time of catch Micrococcus and Flavobacterium predominate on the bodies of these fish species. As spoilage progresses at cool temperatures (0-5^{o}C) these organisms are replaced by Pseudomonas and Achromobacter species.

Characterization Of Spoilage Bacteria

It is clear then than a particular group of bacteria are mostly responsible for the spoilage of freshwater fish. Various workers have found different Pseudomonas species to

be involved in spoilage. Pseudomonas putrefaciens was identified as a fish spoilage microorganism in both salt and fresh water fish by Castell et al. (1949) and by Silverio and Levin (1967), respectively. Pseudomonas fragi was identified by Castell and Greenough (1958) as another fish spoilage microorganism. Studies by Chai and co-workers (1968) indicated that on haddock fillets Pseudomonas putrefaciens and fluorescent Pseudomonads increased at a faster rate than all other proteolytic microorganisms during refrigerated storage. Similar findings were reported by Corlett and co-workers (1965) on their work with dover sole fillets. These findings may also apply to fresh water fish such as Whitefish.

Results such as those published by Lerke and co-workers (1965) indicate that not only is Pseudomonas implicated in the psychrotrophic spoilage of English sole fillets, but they go one step further and identify the specific groups of Pseudomonads which are most responsible for this spoilage. Other workers found similar results with haddock (Power et al., 1968; Levin, 1968; Chai et al., 1968; Boyd and Southcott, 1968), with ocean perch (Lee et al., 1967), and with cod (Regier et al., 1970).

The Lerke group used the classification scheme of Shewan and co-workers (1960) to classify their isolates from plate counts of spoiling fish into four categories of Pseudo-

monas organisms on the basis of biochemical activity and pigmentation (Table 1). They found two out of seventy Group 1 cultures to be capable of spoiling fish. None of the one hundred and fifty-nine Group II isolates were found to be capable of spoiling fish. All forty-nine of the Groups III and IV isolates were found to be capable of causing deteriorative spoilage.

Lerke's group found that only certain species in the Pseudomonas-Achromobacter group, which grew well at 5°C , could spoil their English sole fillets. No spoilage organisms were found in the Coryneforms, Micrococci, or Flavobacteria. About 29% of the Pseudomonas-Achromobacter group were found to be able to spoil the sole fillets. They could not however, find significant biochemical differences between organisms causing spoilage and those which did not, that could be correlated to any degree.

A Vibrio group was found which was fermentative, produced acid, but no gas in glucose, and an Aeromonas group was found which was also fermentative, acid positive, but produced gas in glucose. Both groups were found to be capable of producing spoilage.

Finally, they found that although Flavobacterium could grow well at 5°C , none of the cultures they isolated could spoil fish press juice.

Due to the extremely complex microbiological popu-

TABLE 1

DIFFERENTIATION OF THE PSEUDOMONAS GROUP

PSEUDOMONAS GROUPS	MODES OF DIFFERENTIATION	
	HUGH-LEIFSON TEST	PIGMENTATION
I	OXIDATIVE	DIFFUSIBLE GREEN
II	OXIDATIVE	NO DIFFUSIBLE PIGMENT
III	ALKALINE	NON-PIGMENTED
IV	NEGATIVE	NON-PIGMENTED

*Shewan et al., 1960

lation and its activity, it could be suggested that perhaps the problem of spoilage should be looked at on a higher group type level rather than looking at specific spoilage bacteria.

The Spoilage Process

Immediately after death a complex series of enzymic changes occur in the fish muscle. Shewan (1962) reported that the flesh remains sterile for three to four days at 0^0C. Evidently there are four possible pathways which bacteria may follow in order to enter and degrade the fish muscle. The first pathway involves entry of the organisms through the gill tissues, proceeding along the vascular system, particularly along the caudal vein, through the kidneys, and then after some days, into the muscle tissue. The second pathway involves the penetration of the organisms through the intestines into the body cavity and belly walls. The third pathway involves the entry of the organisms through the skin and into the flesh of the fish. The fourth pathway is the most significant one in considering the spoilage of freshly cut fillets. It amounts to a surface, and in most cases, a subsurface inoculation, of organisms into the exposed muscle tissue during and after handling.

The Antibiotic Properties Of Mucus Slime

Fletcher (1968); Brock (1958); and Braekkan and Boge (1964) all reported that the mucus slime of fish,

although containing thousands of bacteria per ml , has antibiotic properties which prevent the penetration of the surface bacteria to a high degree. Once this protection has been removed and the unprotected tissue is exposed, spoilage will begin to occur.

FISH PRESERVATION: HISTORICAL METHODS (BORGSTROM, 1969)

Salting, Smoking, and Drying

The oldest methods of preserving fish are salting, drying, and smoking. These methods are still in use today. Although they remain basically unchanged, refinements have been made in each process. Sodium chloride and potassium nitrate salts were used in order to give a long shelf-life to a valuable food protein source. Salt fish is more of a delicacy than a staple in most parts of the world today.

As early as 3500 B.C. the river delta civilizations of Mesopotamia, Egypt, and the Indus Valley used dried, salted fish. Salting had a two-fold function. It provided an antiseptic action and lowered the available moisture levels below minimum bacterial requirements.

Smoking of foods goes back into history at least as far as fire does, and was probably used before salt as a preservative. It was most likely discovered that fish hung near the smoke vent of a cave or tent were penetrated by the vapours and smoke with the result that they tasted more interesting than raw or cooked fish. More importantly, they

lasted longer without spoiling, than fish which had been stored elsewhere. The vapours and smoke contain a wide range of chemical compounds. Their exact nature and composition depend upon such things as the type of wood used and the time-temperature factors. The mixture of compounds generally may be said to contain alcohols, organic acids, phenols, aldehydes, ketones, ethers, and hydrocarbons.

In later years, fish were smoked over hardwood and hardwood sawdust fires. This imparted surface sterilization, lowered moisture content, and increased salt concentration, which together with the antiseptic action of the smoke constituents checked microbial spoilage quite well.

Today smoking is used to impart a distinctive flavour and color rather than for its preservative properties. Products such as smoked salmon, whitefish, and herring are traditionally popular smoked fishes.

Drying of fish in the open air is the most ancient method of preserving fish. The Phoenicians relied heavily on air-dried fish as a staple dietary item. This form of preservation has been practised to date by almost every fishing country to some extent. The evaporation of water results in a drastically lowered availability of moisture necessary for bacterial growth and spoilage.

Often a combination of the above methods was used such as drying and salting. This proved to be the most

effective since moisture concentration was greatly lowered while salt concentration was greatly increased. Since most bacteria are inhibited by a 15% salt concentration spoilage was effectively forestalled.

Marinating, Fat-Embedding, And Lime-Curing

Marinating in vinegar dates back to Roman times when this method was used on tuna, mackerel, and perch. The Amazons used a method of fat-embedding to preserve fish. They called it 'mixiria'. Lime-curing of fish was practised in the Middle Ages in Scandinavia with dried pike, cod, and ling. It was used to make dried fish more appetizing and easier to reconstitute.

Freezing And Freeze-Drying

Peoples such as the North American Plains Indians used natural winter temperatures to freeze and preserve their fish. In the sixteenth century the Norwegians 'freeze-dried' their catch by hanging the fish in below freezing fast moving winds.

FISH PRESERVATION: METHODS OF THE INDUSTRIAL ERA

All the previously mentioned traditional methods are still used today to some extent but mostly for the development of flavour and aroma rather than entirely for preservation.

Canning And Heat Processing

Except for the discovery around 1300 A.D. that gut-

ting significantly improved the quality of salted herring, no radically new methods of fish preservation were developed until the ninteenth century. About this time Nicolas Appert invented the process of heat treatment combined with hermetic sealing. The applicability of the canning process depends upon the characteristics of the fish species to be canned. In general, factors such as water content and fat content dictate the final characteristics of fish preserved in this way. Some species such as sardines, salmon, tuna, and herring are particularly suited to this type of preservation process, whereas others such as cod and hake are not particularly suited unless they are specially prepared in sauces or spices, for example (Borgstrom, 1969).

Live Products

Keeping fish alive in the holds of fishing vessels until reaching port was a practise introduced in England in 1712. By the end of the ninteenth century fish pens were common on the Great Lakes for the retention of sturgeon and whitefish in the fall for sale in early winter (Borgstrom, 1969).

Natural Ice, Chilling, And Freezing

From 1805 onwards natural ice was used in the Southern United States. Today approximately half of all fishery products in North America are preserved in ice during transportation and holding for immediate sale in

local markets.

The development of mechanical refrigeration made it possible to keep fish fresh at times when natural ice was not available and also lowered the cost of transport due to weight reduction in the absence or partial lack of ice. Although the first refrigeration apparatus was made in England in 1834 by an American named Perkins, the first practical compressors were not developed until 1874. They used either sulfur dioxide or ammonia as refrigerants.

Natural freezing was first used many centuries ago. Probably the Scandinavian fishermen were the first to use it. Ice-salt mixtures were used even before the advent of the refrigeration compressor apparatus. At the beginning of the nineteenth century ice-salt mixtures were used to hold salmon at much lower temperatures than could ice alone during transport to local markets. In 1868 this process was used in a plate freezer apparatus with the mixture separated from the fish by thin metal pans which held the fish. In 1867 Reece invented an ammonia freezing machine. The first plant for the freezing of fish was erected in New South Wales , Australia, in 1873. Mechanical freezing of fish on board ships was first employed in Astrakhan, Russia, in 1888. The Japanese first attempted freezing of fish commercially in 1901, and by 1923 several of these types of plants were in operation.

The freezing process today is well developed. Much research has been done in order to develop the best

methods of freezing to provide a quality product with as long a shelf life as 6 months. For instance, the best way to freeze fish is to rapidly freeze it. This may be accomplished by various means such as blast freezing, or by fast freezing in liquid gases. For example, liquid nitrogen freezing has a large advantage over cold air freezing in that the loss of weight due to evaporation is less than 1% as compared with 6 to 8% by air freezing. This fast freezing process has the effect of preventing excessive drip loss due to ice crystal disruption of tissue integrity. Cell damage, loss of flavour, and discoloration are reduced or entirely prevented and the nitrogen atmosphere prevents oxidative deterioration. A further advantage of nitrogen freezing systems over conventional systems, is that they take up approximately one sixth of the space normally required for air freezing systems. Glazing also prevents moisture loss during storage. The filleting process itself facilitates faster freezing and easier handling (Borgstrom, 1969).

Tanikawa and Shitamori (1963) indicated that treatment of fish and fillets prior to freezing with polyphosphates considerably reduced drip loss upon thawing and even slightly enhanced texture and flavour over non-treated controls. Vacuum packaged frozen trout and shrimp have proven to be superior in quality than if packaged in other ways.

Chemical Preservation

In addition to the historical methods such as salting, smoking, marinating, fat-embedding, lime-curing, pickling, and fermentation, new techniques have recently been investigated. These techniques usually involve the addition of a chemical to the fish by surface application in the form of a dip or spray. The following list will be limited to chemicals which have been used or could possibly be used for the preservation of fish fillets.

Chlorine and related compounds

The use of chlorine and related compounds either in water solution or in germicidal ices as a fish preservative was reported as early as 1923 by Gibbs. He found that ice containing 0.025% sodium hypochlorite was somewhat effective in controlling bacterial spoilage of whole eviscerated iced fish. Later in 1926, Chen and Fellers published their findings on sodium hypochlorite preservation of fish. By washing fish in solutions of 0.6% available chlorine in the form of sodium hypochlorite spoilage was delayed. However, halibut unfortunately displayed the peculiarity of turning yellow.

Tarr (1961), however, did not recommend the use of chlorine or related compounds that readily liberate free chloride ions. They are practically ineffective in preserving fish owing to the chloride ion's great instability in

the presence of readily oxidizable organic materials such as fish. However, he did recommend that these compounds could be used as sanitation agents in fish plants.

Conflicting reports exist about the reason for the effectiveness of sodium chloride as a preservative. The foremost reason for its preservative activity is because of its dehydrating capacity and its detrimental osmotic effects on most bacterial cells. Some reports indicate that the chloride ion in solution has germicidal activity. However, other reports dispel this claim because of the reasons posed by Tarr as stated above. They affirm that it is the activity of the sodium ion which is germicidal. Whichever is the case, the more significant information which has been gained about the activity of sodium chloride in preserving fish and fish products is that in some species of fish, especially with Whitefish, rancidity is accelerated by the use of sodium chloride and the presence of heavy metal ions. Castell et al., (1965); Banks (1937); Banks et al., (1957); Tarr (1944).

Tarr and Sunderland (1940) investigated the use of sodium chloride with various other preservative compounds. Chemicals such as benzoic acid, para-hydroxy-benzoic acid ethyl ester, sulfur dioxide, hydrogen peroxide, chloroform, potassium nitrate, boric acid, hydrochloric acid, sodium nitrate, and potassium nitrate have been investigated in conjunction with sodium chloride. However, limited success was

found with most of them. The best results were found with nitrates. However, the use of these compounds is becoming increasingly discouraged. The problems of carcinogenic nitrosamines being formed and colour changes occuring in some cases, prevent the use of nitrites on fish (Sen et al., 1970). Nitrites are not permitted on fresh fish in Canada.

Benzoic acid and benzoates

Benzoic acid occurs naturally in cranberries, lingonberries, cloudberries, loganberries, some blackberries, prunes, and green gage plums. They act as natural preservatives and in the first two examples occur frequently in quantities exceeding the maximum limit set for manufacturing processes of 0.1%. The optimum working pH range is between 2.5 and 4.0. Therefore if a food such as fish, which has a pH of about 6.0 is preserved with benzoic acid, the same amount of benzoate added at the different pH will work only about one-hundredth as well as at pH 3.0. Magnesium benzoate often replaces or accompanies the sodium salt (Borgstrom, 1969).

Sorbic acid and sorbates

Sorbic acid has been used extensively in the fruit and fruit preserves industry to great advantage. It has the capacity to inhibit yeast and mold growth at low concentrations. At present a maximum of 0.1% sorbic acid is permitted in fruit preserves, jams, and jellies. This ef-

fectively controls the most resistant and tenacious microorganisms such as the osmophilic yeasts (Borgstrom, 1969).

Sorbic acid is easily metabolized in humans to carbon dioxide and water. It inhibits several species of molds, yeasts, and some bacteria. Its operating mechanism was described by Emard and Vaughn (1952). Apparently it blocks the normal functioning of some sulfhydryl group containing enzymes. The blocking mechanism is selective as it suppresses catalase positive organisms such as most bacteria, actinomycetes, molds, and yeasts.

Recently, work carried out by Devebere et al., (1972) indicated that psychrophiles of the genera Pseudomonas, Micrococcus, and Achromobacter responsible for fish spoilage at low temperatures, could be inhibited by treatment with 0.3% sorbic acid.

Wallhauser and Luck (1972) reported results which indicated that Escherichia coli and Aerobacter aerogenes required lower inhibitory concentrations of sorbic acid than did Staphylococcus aureus and Pseudomonas aeruginosa, with the least effect of all on Lactobacillus arabinosus, of faecal origin. Sorbic acid does not interfere with Leuconostoc spp. and Clostridium spp. and in fact may even promote their growth.

Belgian workers have found sorbic acid to have an inhibitory effect on the production of trimethylamine in

marine fish (Devebere and Voets, 1970). Sorbic acid has been successfully applied as a preservative to sauerkraut, wines, cheese, bread, liquid eggs, fruits, margarines, sausages, shrimps, deep fried fish, and salad dressings. A Japanese patent (1971) makes use of Vitamin B compounds to enhance the effects of sorbic acid in a synergistic manner.

Little work has been attempted on fresh fish fillets using sorbic acid as a preservative. Some work with this chemical has been done on fish fluids and acidified processed fish products. There is a problem in that sorbic acid's inhibitory activity is at a maximum around pH 5.0, whereas the pH of the fish is closer to 6.0. Obviously, if sorbic acid is to be at all effective in forestalling bacterial spoilage of fillets, then a higher concentration and longer exposure time than that used for fruits or acidified fish products may be required.

The legally permitted concentration of sorbic acid in fish is 2,000 p.p.m., up to 3,000 p.p.m. in certain other foods in the United States, and a maximum limit of 1,000 p.p.m. in Canada. To date, no research data has been found, on the treatment of whitefish fillets with sorbic acid as a preservative treatment. One of the possible side benefits to treating fillets with this chemical was elucidated by Ando and Nagata (1971). They found that sorbic acid increased the water binding capacity of the fish muscle.

This activity is reflected in less water loss during fresh storage and even more especially on thawing after having been frozen. Shtenburg and Ignat'ev (1970) carried out a toxicological evaluation of some combinations of food preservatives, one among them being sorbic acid. The chemicals used for the study were nisin (dosage: 2-4 mg/kg animal weight); sorbic acid (40-80 mg/kg); benzoic acid (40-80 mg/kg); sodium bisulphite (80-160 mg/kg); and combinations of the above. From conclusions on short and long term studies on rats and mice it was found that 40 to 80 mg/kg animal weight daily dose of sorbic acid was not harmful while all the other chemicals and combinations of chemicals caused at least some harmful effects. They decided that further restrictions should be placed on the use of benzoate and sulphite and that sorbate should be used, wherever possible, as an alternative.

Propionic acid and propionates

Propionic acid is a product of bacterial enzymic activity on lactic acid during the manufacture of Swiss cheese. It prevents mold growth and contributes to the flavour of the cheese as well. The development of bread mold and rope caused by Bacillus mesentericus is prevented by adding 0.2 to 0.4% calcium and sodium propionate to the flour as a preservative measure.

These compounds are also used to prevent mold

growth in cottage cheese, fruit, vegetables, chocolate and malt extracts, and food wrappers are impregnated with them also. In India, before salting, fish have been dipped in propionic acid solution as a preservative measure (Borgstrom, 1969).

Preservative solutions containing propionic acid and its sodium salt in combination with benzoic acid and its sodium salt have been moderately successful when used in ice or dipping solutions (French patent, 1970). Optimum activity is within the pH range of 3.5 to 4.5. Therefore as it is used presently it would not in low concentrations (eg. lower than 3.000 p.p.m.) have much of a preservative effect on fish which has a more neutral pH. The legal permitted limit is 3800 p.p.m. in the U.S.A. and 3,000 p.p.m. in Canada.

Acetic acid and acetates

Acetic acid has been found to be one of the most efficient weak acids for the preservation of fruits and vegetables. It is used in the manufacture of catsup, mayonnaise, pickles, and other products. It is easily metabolized by humans and is much more potent a germicide at the same pH than both lactic and acetic acids. Sodium diacetate is used to prevent ropiness in bread and is permitted to a maximum residual of 3,000 p.p.m. in Canada (Borgstrom, 1969). Acetic acid has been used to marinate fish such as herring but would

be unsuitable for fresh fish fillets due to its vinegary taste.

Citric acid and citrates

The germicidal activity of fruit juices is due mainly to the presence of citric, malic, and tartaric acids. It has been found that a pre-treatment of fish by a dip in citric acid or sodium citrate solutions improves the keeping quality of subsequently dried fish products such as Indian 'choodai' (Ingram et al., 1956).

Germicidal ices

Various chemicals have been incorporated into ices as an extra preservative measure. Among those compounds used are: sodium chloride, sodium hypochlorite (Gibbs, 1923), sodium benzoate, antibiotics, nitrites and nitrates, and ozone. Antibiotic usage in ice, especially the use of chlortetracycline, has been accepted in North America as a method of keeping fish fresh until reaching port (Frazier, 1967). However, in recent years Canada has disallowed its use, due to public health reasons.

Ozone and ozonated ice and water

Salmon and LeGall (1936) recommended the use of ozone to maintain the freshness of fish. They found that a concentration of 2.0 mg/cm^3 or 0.02 mg/liter was effective in controlling Pseudomonas spp. The disadvantage in the use of ozone is that it is not accepted for use in fish preser-

vation nor is it likely to be because of the possible dangers to human health in its generation if inferior or maladjusted equipment is used, and because of its strong oxidative properties.

Hydrogen peroxide

Metzner et al.,(1938) reported the first usage of hydrogen peroxide to preserve whole fish and fillets. They used a 0.3% solution of hydrogen peroxide. Lucke (1938) however, disagreed with this treatment because of the fact that it was merely surface active and gave a misleading idea of the real quality of the fish. Tarr and Sunderland (1940) found that hydrogen peroxide had only a transient effect on fish and the microorganisms which caused spoilage. This was presumably because the muscle catalase rapidly decomposed it.

Hexamethylenetetramine

Hexamethylenetetramine is used in Scandinavia and Continental Europe as a fish preservative (Borgstrom, 1969). In order to be activated it must first be hydrolytically split in acid media. This hydrolysis reaction forms formaldehyde, a natural constituent in many fish and fish products. It is used in concentrations of 0.02 to 0.25% and cannot be considered toxic in these concentrations. $L.D._{50}$ concentration for rats is 9.2 gm/kg . Bacterial growth is suppressed at a concentration of 0.05% at pH 7.0 , yeasts by 1.0% , and molds by 5% .

This chemical actually kills microorganisms by forming methylene bonds by reaction with proteins. This induces hardening, and therefore treatment of tissue with it is not entirely satisfactory from a textural viewpoint.

Formic acid

Formic acid, a natural constituent of currants, grapes, and honey, has been used as a preservative for holding fish until processing. In concentrations of 0.15 to 0.2% the growth of yeast, molds, and lactic acid among other bacteria are suppressed. Formic acid partially inactivates lipases, peroxidases, and dehydrases, while catalases are more fully suppressed (Borgstrom, 1969).

Nitrites

Nitrites were first used as a colour fixative and not as bacteriostats. Nitrites are able to inhibit some bacteria but are much less able to inhibit common Lactobacilli and Streptococci, which are rather resistant. The maximum allowed level of nitrite in food is 200 p.p.m. but levels are usually found at from 75 to 175 p.p.m.

Tarr and Sunderland (1940) found that 0.1% potassium nitrite was excellent for the retardation of bacterial growth. It was also good because nitrites were found to disappear by reduction to ammonia by certain bacteria. Unfortunately this disappearance is dependent upon the amount of nitrite reducers present, which varies.

Yamada and Murata (1949) found that the use of

0.02% sodium nitrite in conjunction with 5% sodium chloride and ice, inhibited bacteria and trimethylamine production. The action of sodium nitrite is pH dependent with no effect over pH 6.0, however pH 5.7 (200 ug/ml) is the pH at which optimum inhibition occurs. That pH is close to the normal condition in fish muscle.

Unfortunately there are serious disadvantages to the use of nitrites in preserving meat or fish. It has been proven that nitrites have a pro-oxidant activity on frozen fish, and they are mildly antioxidant in unfrozen fish. Sen et al. (1970) demonstrated that a more serious disadvantage is that nitrosamines are formed in nitrite treated fish. They showed that the sodium nitrite reacts with various methylamines present in fish to give nitrosamines, such as dimethylnitrosamine, which are potential carcinogens.

Sulphur dioxide

Tarr and Sunderland (1940) reported this chemical as an unsuitable preservative in the case of fish as it apparently affects a reduction of trimethylamineoxide to trimethylamine and has detrimental effects on Vitamin B_{12}.

Antibiotics

Chlortetracycline is obtained from Streptomyces aureofaciens, oxytetracycline from S. ramosus. Both antibiotics are used in many countries, among them the U.S.A., U.S.S.R., and Japan, for holding fish in ice until proces-

sing. The maximum permitted level is 10 to 15 p.p.m. The use of antibiotics is recommended only with the use of chill temperatures as close to $0^{o}C$ as possible. The danger is that as the lower temperature limit is passed, pathogenic organisms resistant to the antibiotics become dominant and constitute a health hazard. The genetic development of new resistant strains of human pathogens is also a dangerous possibility. Therefore anitbiotics cannot seriously be considered as a feasible long term alternative to other preservative chemicals which may be just as effective.

Approximately 5 to 10% of the population is allergic in some degree to some or all of the antibiotics. This presents marketing problems and therefore use of these agents is declining (Tarr, 1961).

There has been considerable research done on the use of antibiotics in fish, however it is not the writer's intention to go into the topic in any more detail than described above. Papers of interest on this topic includes: Lee et al., (1967) and Boyd and Southcott (1968).

Antioxidants

Borgstrom (1969) reported that gentisates such as 2, 5-dihydoxybenzoic acid or gentisic acid have been used with considerable success in improving the flavour and keeping quality of meats and fishes because of their solubility in water and their overall effectiveness. Ethyl gallate has

proven to be an effective antioxidant for salmon and herring but its use will soon be prohibited in North America. Ascorbic acid has a synergistic effect in combination with other antioxidants and is a useful additive to fatty fishes in the prevention of rancidity. In Russia both butylated hydroxyanisole and butylated hydroxytoluene have been successfully applied to fish as antioxidants in the form of heated aerosols. EDTA salts and related compounds have received a considerable amount of attention in recent years as food antioxidants and also as preservatives of oxidizable fats, oils, etc. For example, it has been found that in Salmon muscle 100 to 500 p.p.m. of disodium EDTA effectively retards oxidative rancidity and subsequent odour formation. Similar studies by Boyd and Southcott (1968) with other fatty fish have shown similar results. These workers dipped Pacific cod fillets in 1% EDTA solutions. The calcium disodium salt was found to be ineffective in forestalling spoilage, however, the disodium salt was found to be somewhat effective. Pelroy and Seaman (1969) dipped sole and ocean perch in 1% EDTA solutions. They obtained a 7 to 10 day shelf life extension with disodium EDTA treated fillets stored in vacuum packed plastic bags. The treatment with the calcium salt was less effective with only a 4 day extension in shelf life. They found that the extensions were due to the inhibition of Pseudomonas spp. Power et al., (1968) treated haddock filletts with three solutions of EDTA salts. They found no ex-

tension of the shelf life with the calcium disodium salt, an 11 day extension with tetrasodium EDTA, and a 6 day extension with disodium EDTA. Regier et al., (1970) treated cod and haddock fillets directly off a commercial filleting line with various EDTA solutions in the form of a spray. They found little effect on the overall numbers of bacteria. However, they observed a decrease in Achromobacter spp. originally present in large numbers, and an increase in Pseudomonas spp. originally present in smaller numbers. Pseudomonas became the dominant group by the time spoilage was evident. On further examination they discovered that sharp changes were occurring within the Pseudomonas group itself. Castell et al. (1949) found that Pseudomonas putrefaciens, which has been implicated as a particularly active spoilage organism, was suppressed by the treatments with the sodium and calcium disodium salts of EDTA. Again, the sodium salt was proven to be more effective than the calcium disodium salt. Gray and Wilkinson (1965) showed that EDTA is bactericidal for Pseudomonas aeruginosa with 0.0001M destroying over 99.99% of the cells in a suspension. Castell (1949) found that Pseudomonas Groups I and II were not sensitive to the EDTA treatment and Group II proliferated in spite of the presence of EDTA while Group I neither increased nor decreased. Goldschmidt and Wyss (1966) attributed the toxicity of EDTA to complexes formed between EDTA and nitrogen compounds. Levin (1967) found that the proportion of Pseudomonas put-

refaciens and Pseudomonas fluorescens of the total spoilage flora was not significantly changed between untreated and EDTA treated haddock fillets. However their initial growth response was markedly affected. He also found that EDTA treated haddock fillets showed improved odour and taste scores over controls.

There is a possibility that the EDTA could tend to decrease the available calcium in certain foods through chelation and thus partially destroy the nutritional value of the food. It is for this reason that public health advisory agencies are recommending that continuing research with EDTA be done with the calcium or calcium disodium salts of EDTA rather than the sodium salts. The use of the calcium salts of EDTA minimizes the danger of calcium removal from the food through chelation. Unfortunately since it is the chelating action of EDTA which is responsible for the inhibition of bacterial enzyme systems, a reduced calcium chelating capacity also affects the ability of the treatment to inhibit bacterial spoilage. A 300 p.p.m. residual of disodium EDTA could chelate 13 to 40% of the available calcium present in a cod or haddock fillet. Toxicity studies with rats fed EDTA salts found no toxic effects with the calcium disodium salt over one year and with the disodium salt over a twelve week period (Chan and Yang, 1964).

Miscellaneous compounds and mixtures

A United States Patent (1971) reported that t-

butylhydroperoxide was effective against bacteria which cause low temperature spoilage (around 4°C) of fish in concentrations of from 20 to 30 p.p.m.

A Japanese Patent (1970) reported that water bleached, finely divided fish meat mixed with certain amino acids and sorbitol prior to freezing and storage undergoes minimal protein degradation.

Another United States Patent (1970) described aqueous compositions containing certain 2-OH-polychlorodiphenyl ethers and polyalkylene polyaminopolyacetic acid salts as having been successful in preserving fish.

Natural antimicrobial factors in fish

Brock (1958) and Braekkan and Boge (1964) found that protamines such as clupeine and salmine which are abundant in the milt of fish have antimicrobial characteristics. These antimicrobial factors control and limit bacterial growth in fish and make it impossible for bacteria to penetrate the epidermis under normal conditions. These antimicrobial factors may possibly be purified from fish milt during the washing procedure and added back onto the washed fish as a preservative. No workers seem to have yet investigated this possibility.

Controlled Atmospheres

The effects of controlled atmosphere application to fish as a preservative technique have been well outlined

by various workers Shaw and Nicol (1969); Shewan (1949); (1970); Michael et al., (1968); Durrant (1969).

Shewan (1949) described controlled atmospheres of 95% carbon dioxide as good for the control of bacteria on fresh fish. The major disadvantage to its use was that it had an objectionable effect on the appearance of the fish. He reported similar effects in 1970. Carbon dioxide was applied through dry ice in closed containers and they were then flushed with carbon dioxide to remove the remaining air. Michael and co-workers (1968) found that commercial haddock fillets stored at 3°C in 0, 30, 70, or 100% carbon dioxide all showed an increase in shelf life over non-treated air controls. A sharp increase of twelve days over the air treated samples shelf life of five days was obtained with 100% carbon dioxide treatment.

The gram-negative group of bacteria, predominantly Pseudomonas, Achromobacter, Alcaligenes, and Vibrio-Aeromonas Group, were almost completly suppressed by storage in carbon dioxide. This evidence is supported by the findings of Shaw and Nicol (1969). They found that at 5°C non-pigmented Pseudomonas were not inhibited over the range of 0.8 to 100% environmental oxygen. However, at 0.2% oxygen the growth rate was one quarter of that in normal air. They did not grow at all in the absence of air. Pigmented Pseudomonads were inhibited by 10% carbon dioxide. Inhibition was found to be independant of oxygen concentration above 1% oxygen.

Micrococcus was not inhibited by 10% carbon dioxide. The inhibition mechanism was unclear but perhaps it could have been due to an unfavourable decrease in pH or by specific inhibition.

Michael and co-workers (1968) found that carbon dioxide favoured the growth of Lactobacilli, Streptococci, and some Micrococci. They found that carbon dioxide combined with gamma irradiation of 100 krad was effective in eliminating Pseudomonads completely (See Irradiation Preservation).

Durrant (1969) reported on the use of carbon dioxide saturated brines to preserve fish or as a holding procedure. However, he pointed out that there was a potential danger in that anaerobes could grow and utilize sulphur containing amino acids to produce poisonous hydrogen sulphide.

Other workers report that even though carbon dioxide may successfully extend shelf life from a microbiological standpoint, unfavourable changes in colour and texture occur in some fish species treated in this way.

Irradiation Preservation

Various workers have found low level gamma irradiation to be somewhat effective in controlling the growth of bacteria on fish and fish products. The general rule of thumb seems to be that the higher the level of irradiation the more pronounced is the effect of kill on microorganisms.

However, as the level of irradiation increases the deleterious effect of irradiation on flavour increases.

Diehl and co-workers (1969) found that radiation 'pasteurization' can reduce bacterial counts by a factor of 10^4 to 10^7 bacterial numbers per gram of fish, with subsequent refrigeration. A radiation dose of 0.5 Mrad can double or triple the maximum cold storage life of meat, chicken, fish, and strawberries. Meat is peculiar in that it may acquire an unpleasant taste when subjected to the required treatment unless it is carried out below $-20^{o}C$. Irradiation sterilization below $-90^{o}C$ usually will reduce the deleterious effects almost wholly.

Michael and co-workers (1968) found that commercial haddock fillets treated with gamma irradiation and stored in 100% carbon dioxide were preserved an extra seven days over controls which were merely treated with 100% carbon dioxide controlled incubation atmosphere. Pseudomonas, Achromobacter, Alcaligenes, and the Vibrio-Aeromonas Group were almost completely suppressed by storage in carbon dioxide while the irradiation eliminated the Pseudomonads completely.

Liston and Matches (1969) found 50 Krad irradiation to have no effect on the extension of shelf life of very fresh low count fish stored at $1^{o}C$ in polyethylene bags. They packed Whitefish in plastic films impermeable to oxygen and water and treated their samples with radiation at 100 Krad. They then stored them in wet ice for six weeks. Sen-

sory, chemical, and microbiological tests revealed that the 100 Krad treatment extended the shelf life of the fresh whitefish from nine days for the untreated controls to twenty-three days for the treatments. They found that 100 Krad reduced the total bacterial population by approximately 70% compared with untreated fish, while doses of 200 to 400 Krad decreased counts to less than 100 organisms per gram.

Miscellaneous Preservative Techniques

Microbiological inhibition of specific spoilage bacteria

Price and Lee (1969; 1970) reported that Lactobacilli from marine sources which produced hydrogen peroxide could inhibit the growth of Pseudomonas and prevent or at least delay subsequent spoilage. They also found that Lactobacillus plantarum inhibits the growth of Pseudomonas, Bacillus, and Proteus, with Pseudomonas being the most sensitive. Peak production of hydrogen peroxide in these Lactobacillus species occurs in four to five days at 30°C.

Bacteriophages

Delisle (1969) found 180 bacteriophages active against psychrophilic fish spoilage bacteria. They were isolated from sewage, fish pier water, and haddock fillets. Most of them formed plaques at 2°C or 20°C and attacked either marine or terrestrial bacterial forms. Most of them were not strain specific or species specific and therefore could not be used for phage typing of the psychrophilic Pseudomonads or for differentiation between marine or terrestrial

types. It is however, feasible to use bacteriophages for the identification of Pseudomonas putrefaciens because species specificity has been demonstrated in this case. In any case, if a preparation of bacteriophages were to be applied to fish as a preservative treatment it could possibly work quite well. A great quantity of bacteriophage would not be required as they would generate themselves.

Packaging

New methods of packaging are being developed every year. Currently the most successful packaging material is petroleum based plastic film. Polyester films which are somewhat gas permeable are recommended for foods such as whitefish because of their low water vapour transmission rate and low oxygen transmission rate. Dessication and oxidation are kept to a minimum and the product keeps much longer especially during frozen storage. Many other plastic films have been developed and successfully used for various applications. Film characteristics vary usually in gas permeability, water transmission rate, and other physical characteristics such as opacity, pliability, strength, etc.

Coatings

A coating acts as a form of packaging. It surrounds the food item with a protective layer of some substance. Coating of fish is a relatively new technique; coating of the packaging material is not new. A West German patent application (1971) describes the use of monoglycerides and di- and

triglycerides mixtures to coat and preserve fish. Any substance that is fairly resistant to bacterial attack which could be applied as in a form of spray and which could also be easily removed or digested by humans, could be used as a protective coating provided it is non-toxic and does not affect organoleptic properties adversely.

Glazing

Glazing is a form of adding a protective coating. It is done after freezing fish in order to prevent water loss and changes in flavour and colour. It may be applied either in the form of a fast cold water dip or preferably as a spray (Jadhav and Magar, 1970).

Washing with surfactants

Successful tests have shown that washing with 'Tween 80' nonionic detergent (0.2%) in water will remove considerably more slime from fish than the same amount of water without 'Tween 80' in an equal amount of time (Haraguchi and Iimori, 1970; Gillespie and Ostovar, 1971). Their reports indicate that the removal of the slime layer by a five minute water wash with 'Tween 80' results in a hundred times lower initial count and after one day's storage at $3^{o}C$ the counts are twenty times lower than controls which were washed in plain water.

SCOPE OF THE INVESTIGATION

This study was initiated to compare the effectiveness of potassium sorbate, disodium EDTA, and their combination in preserving fresh Whitefish fillets. Specifically, the following were investigated.

1) The determination of an effective preservative treatment concentration of potassium sorbate.

2) The determination of an effective preservative treatment concentration for disodium EDTA.

3) The determination of an effective preservative treatment concentration of a combination of EDTA and potassium sorbate.

4) The determination of the organoleptic profile of the treated fillets.

MATERIALS AND METHODS

SELECTION AND TREATMENT OF FILLETS

The Whitefish fillets used in this investigation were obtained directly from the Freshwater Fish Marketing Corporation Processing Plant at Transcona (Winnipeg) Manitoba. Samples were usually taken off one of the regular filleting lines. The fillets were washed at the FFMC, placed in polyethylene film, covered with ice, packed in a corrugated cardboard carton, and transported by automobile to the University of Manitoba Food Science Department for treatment and analysis.

The Whitefish came from a Northern Saskatchewan lake. Ten ounce fillets taken from fish three days old in ice were cut aseptically into 25 gm pieces approximately 2 cm thick using flame sterilized surgical scissors. Fillets and fillet pieces were kept cold on crushed ice until all pieces were cut, added to treatment baths, removed and placed in storage containers. The pieces were dipped into various concentrations (0.01-10% w/v) of the treatment chemical for varying exposure times (10 sec to 7 min). Potassium sorbate and the disodium and disodium calcium salts of EDTA were dissolved in distilled water using weight per volume ratios for the prescribed concentrations.

Control fillet pieces were dipped in distilled water for the same length of time as the maximum chemical

exposure treatment time. This was done in order to equalize the washing effects of dipping in the chemical treatment solutions. Controls provided information on fillet-to-fillet variation and variation within the same fillet.

The treatment baths were constructed of stainless steel. The amount of treatment solution used was approximately ten times in volume the total weight of fish pieces added. For example, for 600 gm of fish pieces, 6 liters of solution were used. Water temperature during treatment was approximately 5°C. In later studies, 500 ml glass beakers were used as treatment baths.

The first series of range finding experiments used a random selection of fillet pieces for treatment. In later experiments, as accuracy became more crucial, paired sample and incomplete block design testing were employed. In the latter cases the treatment baths were 500 ml glass beakers, filled with 250 ml of the treatment solution. One 25 gm fillet piece was added to each bath for treatment. The baths were not used twice.

After treatment each individual piece was placed in a sterile plastic petri plate and stored in a cold temperature incubator at 3°C. They were stored for specified times and then were withdrawn to undergo odour examination and microbiological analysis.

MICROBIOLOGICAL EXAMINATION OF FILLET PIECES

Total Psychrotrophic Population

The 25 gm pieces were removed from cold storage at regular intervals for quantitative microbiological analysis. These values were expressed as $\log_{10}$ of the viable numbers of psychrotrophic bacteria per gram. The values were used to form grwoth curves from which predictions could be made on when spoilage would probably occur or when it had actually occurred. The samples were removed from the petri plates and placed in 16 oz Canadian Mason Jars containing 225 ml sterile phosphate buffer (APHA, 1967) precooled to approximately 3^{o} C in order to prevent heating of the contents during blending. Sterile Osterizer blender units were then screwed on and the samples were blended for two minutes at the 'liquify' setting. The jar, phosphate buffer, blade, gasket, and cap were previously sterilized as one unit. A 10-speed Osterizer blender was used for blending. The homogenate was serially diluted in 99 ml milk dilution bottles containing 99 ml precooled sterile phosphate buffer. The pour-plate method (APHA, 1967) was used to grow the inoculum and Standard Plate Count Agar (Difco) was used as the growth medium. Yeasts and molds were monitored (APHA, 1967) by pour-plating with acidified Potato Dextrose Agar (Difco). The psychrotrophes were inoculated onto SPC agar and were incubated at 3^{o} C for eleven to thirteen days in

order to allow for visible colony production. Petri plates containing between 30 and 300 colonies approximately 1 mm or larger in diameter were counted and results recorded as viable psychrotrophic bacterial numbers per gram of Whitefish fillet tissue. These numbers were then changed to $\log_{10}$ values for the purpose of plotting growth curves.

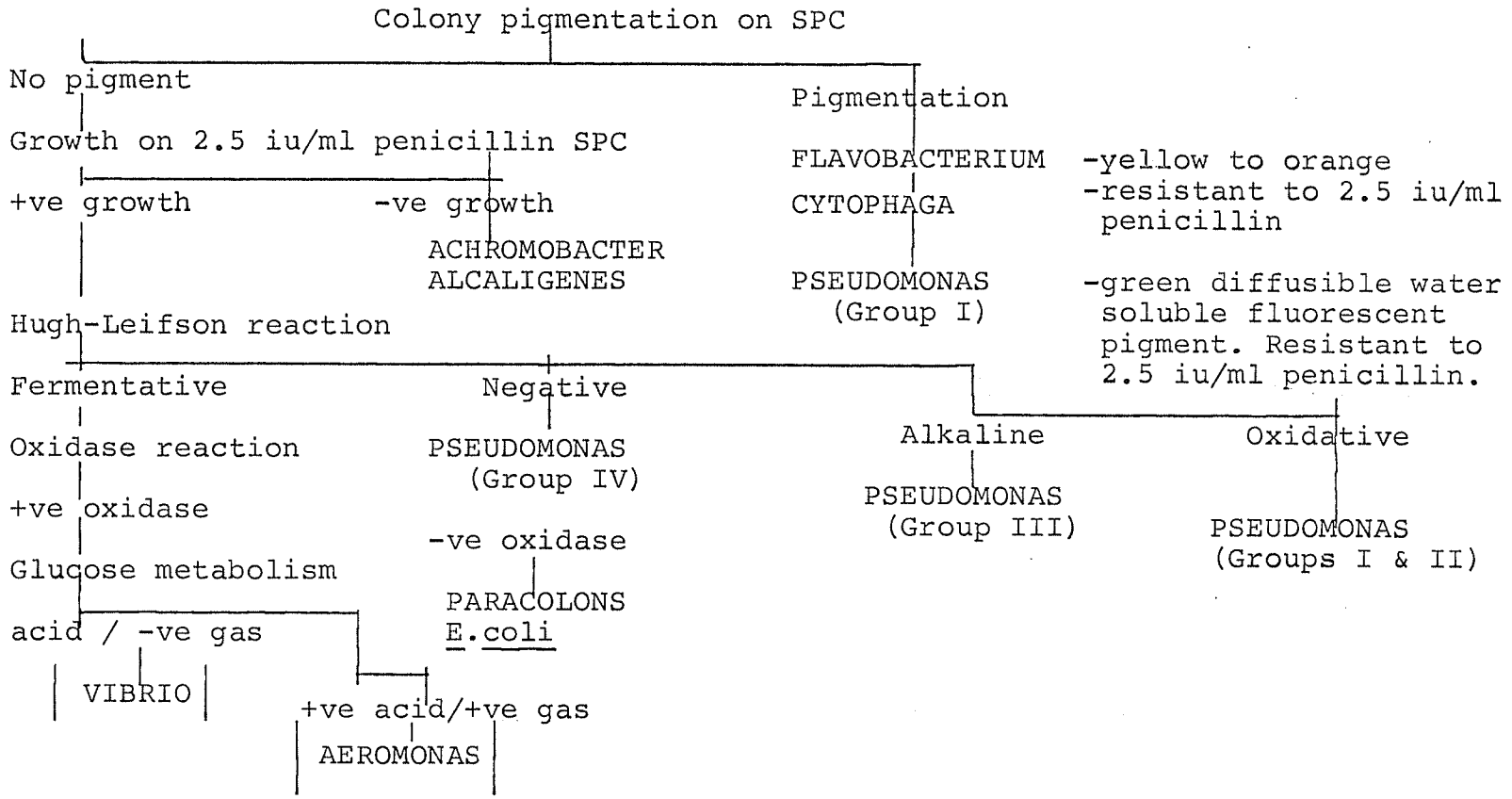
Lerke and Farber (1969) found that a direct viable bacterial count used as a freshness indicator, correlated well with fish freshness tests using the Spinelli (1964) hypoxanthine estimation technique. Although the latter test is the only real test of freshness presently used, it involves a long and complex procedure. A simpler method of freshness determination was required. Since the psychrotrophic bacteria are the main cause of fish spoilage at refrigeration temperatures, a viable count of the psychrotrophic bacteria was chosen to be used as the freshness indicator. Counts reached a level of 10^8 to 10^9 organisms per gram before spoilage of controls became obvious, organoleptically. They were at a level of 10^3 to 10^4 viable cells per gram, initially.

Generic Grouping Of Psychrotrophic Bacteria

In order to determine how the bacterial flora on the fillets were changing with time and according to the type and strength of treatment, the classification scheme of Shewan et al. (1960)(Figure 1) could have been used. However, since it had already been determined that Pseudomonads were

FIGURE 1

IDENTIFICATION FLOWCHART OF THE PSYCHROTROPHIC BACTERIA COMMONLY FOUND IN FISH



primarily responsible for spoilage of controls, the main interest lay in how well the preservative treatments would inhibit Pseudomonas spp. growth. Only a primary indicator test was required for this purpose. The resistance sensitivity to 2.5 iu/ml penicillin-G was therefore chosen as a means of testing for Pseudomonas numbers.

Ten mg of penicillin-G with an activity of 1590 iu/ml was added to 100 ml distilled water, mixed to dissolve the penicillin, then passed through a 0.45 um millipore filter in order to sterilize the solution. Approximately 1.6 ml of this solution was added to every 100 ml of SPC agar media in order to obtain the proper concentration of 2.5 iu/ml. Standard plate count plates with penicillin-G were poured at the same time as the regular standard plate count plates and were used to group the psychrotrophes into two distinct groups, those sensitive to 2.5 iu/ml penicillin and those which were resistant. Standard plate count plates with penicillin-G were incubated at the same temperature ($3^{o}C$) as the regular standard plate counts for the same incubation time (11 days) in order to allow for colony (at least 1 mm) production. Figure 1 shows the major bacterial groups commonly encountered on cold freshwater northern lake fish fillets stored at $3^{o}C$ according to sensitivity to 2.5 iu/ml penicillin-G.

The bacteria in the resistant group were further

subdivided on the basis of reaction in Hugh-Leifson's medium containing lactose (4.0%), sucrose (4.0%), and glucose (4.0%). Table 2 shows this grouping.

Micrococcus and Corynebacterium were not tested for as it had been previously proven that:

i) Micrococcus will not grow in fish juice incubated at 5°C, although they will grow profusely at 15°C.

ii) Coryneforms although able to grow at 5°C have not been implicated in normal spoilage (Lerke et al., 1965). They are usually only detected at the beginning of a storage run and the growth of spoilers usually vastly overshadows the numbers of these organisms.

Table 2 shows that the only organisms found in significant numbers on fish, which are penicillin resistant at 2.5 iu/ml, are the Pseudomonads, Flavobacterium, and Cytophaga. Vibrio, Aeromonas, Paracolons, and E. coli are not present in significant numbers. Since Flavobacterium and Cytophaga are pigmented yellow to orange and Pseudomonads are either colorless or green pigmented there is primary differentiation to roughly establish the major spoilage genera. If mainly composed of Pseudomonads, counts on SPC-penicillin-G agar would be close to counts on regular SPC agar and colonies on the SPC agar plates will be mainly non-pigmented of the Pseudomonas putrefaciens type. It was not necessary to further examine bacterial genera as the study was designed mainly

TABLE 2

DIFFERENTIATION OF THE PENICILLIN RESISTANT GROUP OF PSEUDOMONAS AND OTHER GENERA ON THE BASIS OF THE HUGH-LEIFSON TEST

PSEUDOMONAS GROUPS AND OTHER GENERA	MODES OF DIFFERENTIATION	
	HUGH-LEIFSON TEST	PIGMENTATION
PSEUDOMONAS:		
I	OXIDATIVE	DIFFUSIBLE GREEN
II	OXIDATIVE	NON-PIGMENTED
III	ALKALINE	NON-PIGMENTED
IV	NEGATIVE	NON-PIGMENTED
FLAVOBACTERIUM	OXIDATIVE	YELLOW
CYTOPHAGA	OXIDATIVE	YELLOW
AEROMONAS, VIBRIO, PARACOLONS, *E.coli*	FERMENTATIVE	NON-PIGMENTED

to accomplish two things: a reduction of bacterial growth resulting in spoilage, and concurrently delaying the formation of odour and spoiled flavours due to bacterial and chemical spoilage factors.

A tabulated summary of experiments follows:

CHEMICAL USED	CONCENTRATION	EXPOSURE TIME	APPENDIX TABLES	TRIALS
Potassium sorbate	0.05-5.0%	10 sec-5 min	1-4	1
	1.0%	3, 5, 7 min	7	1
(OC)	4.0%	5 min	8,9	4
(C)	5.9%	5 min	10,11	4
(OSCP)	6/8/10%	5 min	18,19/20,21/ 22,23	2
(OP)	8.0%	3, 5, 7 min	15,16	2
(OC)	9.0%	5 min	12	3
$EDTANa_2Ca$	0.5-1.5%	5 min	5	1
(O)	4.0%	5 min	6	2
$EDTANa_2$ (OSC)	4.0%	5 min	14	2
(OSCP)	6/8/10%	5 min	18,19/20,21/ 22,23	2
(OP)	8.0%	3, 5, 7 min	17	1
$EDTANa_2$/potassium sorbate				
(OSP)	6/8/10%	5 min	18,19/20,21/ 22,23	2
(O)	4% $EDTANa_2$/ 9%potassium sorbate	5 min	13	3

Bacteriological experiments are indicated by the concentrations and exposure times. Odour tests (O) had the same number of trials as bacteriological tests. Sensory tests (S) were composed of duplicate samples or pairs. Chemical residual tests (C) were the averages of duplicate samples. Penicillin resistant bacteria enumeration (P).

SENSORY EVALUATION OF TREATED FILLET PIECES

Odour Investigations

Sensory examination of the controls and treated raw fillet pieces consisted of taste, odour, aroma, and texture determinations in addition to colour, overall appearance and acceptability examinations. Odour was an important test in that without even examining the other factors, the uncooked fish could be judged as either fresh or not fresh. Raw fillet pieces were selected randomly and submitted to independent taste panelists in order to rate these pieces before they were homogenized for bacteriological and chemical analysis.

An objective odour rating scale with number ratings of from one to four (Table 3) was selected in order to apply some type of scale to the spoilage of the fish from an odour viewpoint. The same type of number scale was used for the other organoleptic tests (Figure 2).

Organoleptic Investigations

Texture, general appearance, aroma, and taste of baked fillet pieces were all examined and tested by comparison with fresh untreated cooked fillet pieces. The fish fillet pieces were individually wrapped in pieces of aluminum foil, labelled with a felt pen, and baked at 177^{o}C for fifteen minutes in the middle section of a kitchen type electric range.

Panelists were seated on stools in separate booths

TABLE 3

OBJECTIVE ODOUR RATING SCALE OF WHITEFISH FILLETS

RATING NUMBERS	RATINGS DESCRIPTIONS
1	No noticeable odour, very fresh appearance
2	Very slight odour, a bit fishy, or slightly musty or fruity, acceptable
3	Slightly sour, spoilage has become apparent, fillet pieces are no longer completely acceptable
4	Very sour to putrid, fillets are not at all acceptable as spoilage has progressed too far

Name:

FIGURE 2

Date:

WHITEFISH TASTE PANEL FORM

Instructions:

Do not swallow fish pieces. Eat a cracker and sip water between tastings. Adjust your samples' values by comparison with 'fresh' controls. Ratings are based on two ratings of acceptable in four categories and two ratings of unacceptable. They are 1 & 2 and 3 & 4, respectively as below:

DESCRIPTIVE CHARACTERISTICS

TEXTURE: 1, 2,(whole, solid)
3, 4,(weak, tough, soft, mushy)

APPEARANCE: 1, 2, (normal)
3, 4, (abnormal)

TASTE: 1, 2, (clean, fishy, slightly sweet)
3, 4, (unclean, bitter, sour, rancid, sweet, putrid)

AROMA: 1, 2, (clean, fishy)
3, 4, (unclean, sour, rancid, putrid)

CODE #s	RATING	TEXTURE	RATING	GENERAL APPEARANCE	RATING	AROMA	RATING	TASTE

under controlled light and air flow. Panelists were instructed to eat unsalted soda crackers and sip water before testing began and between all subsequent samples. They were all given identified fresh untreated controls in order to be able to compare them with treated samples. Some of the panelists were given unidentified fresh controls periodically in the course of the many panels in order to check their judgement accuracy. Samples to be tested at each taste panel were randomly selected from the population in frozen storage and distributed four pieces to a judge. Fish pieces treated in the range of what was determined to be optimum levels of treatment were submitted to the taste panel in order to determine the organoleptic effects of some of the treatments.

Four rating categories were used to describe the acceptability. The first two (1 and 2) were acceptable ratings, excellent and good, respectively. The second two (3 and 4) were unacceptable ratings, fair and bad, respectively. Figure 2 shows the taste panel sheet which gives a breakdown of the descriptive qualities of each category. Table 3 shows the descriptive qualities of the odour ratings.

The categories one to four were assigned the number ratings one to four. The ratings for samples which were in the category four which were judged to be actually worse than this rating were assigned the rating of 4+ for the purpose of later decision making on approximate shelf life ext-

ensions. Although the 4+ rating did not weight controls more than a 4 rating these plus ratings were taken into account in some value judgements when comparisons for 20 days storage time were made among controls and treatments.

CHEMICAL EXAMINATION OF FILLET PIECES

Colorimetric Assay For Sorbic Acid Residuals In Fillet Pieces

The method of Nury and Bolin (1962) which they used to determine potassium sorbate in dried fruits, was adapted for use in fish. The method involves the use of the oxidation of sorbic acid to malonaldehyde, which reacts with the 2-thiobarbituric acid to form a red pigment that is measured spectrophotometrically (Figure 3).

The absorption spectrum for potassium sorbate in fish is shown in Figure 4. The recovery of potassium sorbate by the colorimetric method is very similar to what was expected in comparison with those results obtained by Nury and Bolin. Figure 5 shows the standard curve obtained for potassium sorbate in whitefish.

Reagents required

The reagents required for the analysis were Thiobarbituric acid solution, potassium dichromate-sulfuric acid solution, and potassium sorbate solution. The thiobarbituric acid solution was made up by dissolving 0.5 gm of 2-thiobarbituric acid in 20 ml of distilled water and 10 ml

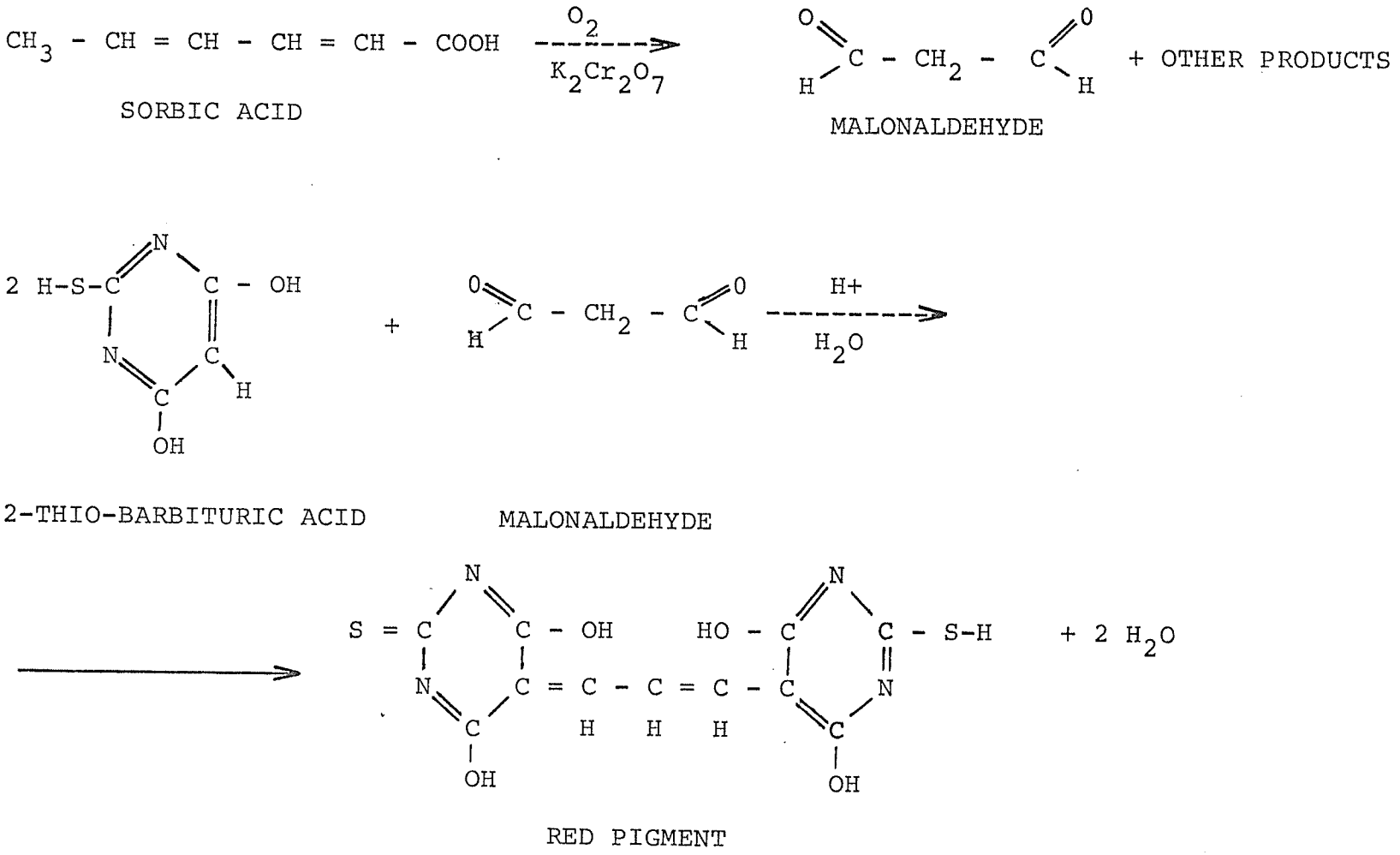


FIGURE 3 COLORIMETRIC SORBATE REACTION

*Sorbate is converted to sorbic acid and is subsequently oxidized to malonaldehyde, which reacts with 2-thiobarbituric acid to form a coloured compound which is measured spectrophotometrically.

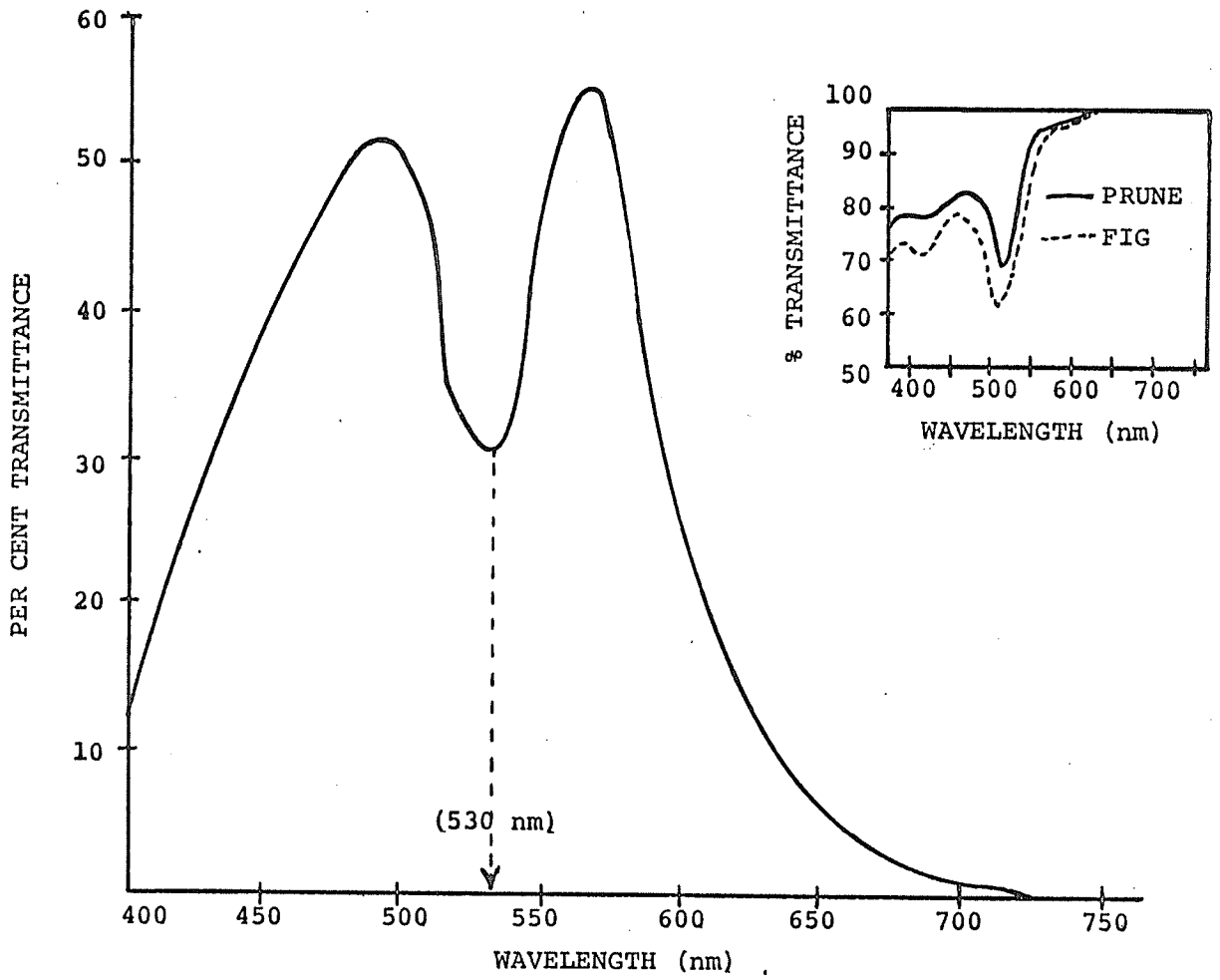


FIGURE 4 ABSORBTION SPECTRUM OF THE THIOBARBITURIC ACID-MALONALDEHYDE PIGMENT IN WHITEFISH

of 1N sodium hydroxide in a 100 ml volumetric flask. Eleven ml of 1N hydrochloric acid solution were added after dissolution of the above, and the solution was made up to volume. The 2-TBA solution had to be made up fresh for each analysis as it would remain stable for approximately 6 hours; after that time absorbance values became erratic and results would have been inaccurate. The potassium dichromate-sulfuric acid solution was made up by combining equal volumes of 0.01N potassium dichromate and 0.3N sulfuric acid. The standard potassium sorbate solution was made up adding 250 mg of potassium sorbate to a 250 ml volumetric flask and making up to volume with distilled water.

Standard curve determination

A 20 gm untreated fish piece was blended in 180 ml distilled water and liquified on a ten-speed Osterizer. Ten ml of slurry was added to each of eight 250 ml volumetric flasks. Standard sorbate solution was added in the amounts of 0, 0.10, 0.25, 0.5, 0.75, and 1.00 ml to each of the volumetric flasks numbered 1 to 6 so that each ml from the 6 different flasks would contain 0; 0.4; 1.0; 2.0; 3.0 and 4.0 ug of potassium sorbate respectively. The two other flasks had 0.25 and 0.75 ml standard sorbate solution added to them and in addition an equal amount of standard disodium EDTA solution (1 mg/ml) was added. These last two flasks acted as controls in order to determine if disodium EDTA would interfere with the sorbate analysis. All the volu-

metric flasks were then made up to volume with distilled water. The contents of each of the eight flasks were then filtered through Whatman #4 filter paper to remove particulate matter and 2 ml of each filtrate was added to each of the eight test tubes containing 2 ml of the potassium dichromate-sulfuric acid solution, respectively. The test tubes were heated for 5 minutes at 100°C in an oil bath. Two ml of the 2-thiobarbituric acid solution was added to each of the eight test tubes and the tubes were then allowed to remain in the oil bath for an additional ten minutes. The tubes were then removed and cooled quickly in running tap water. The coloured solution after cooling was stable for at least half an hour. The absorbance of the coloured solutions was measured at 530 nm in a Spectronic 20 spectrophotometer using water as a blank. Absorbance vs ug potassium sorbate was plotted (Figure 5).

Sample residual determination

The samples were prepared exactly as above with two exceptions. No potassium sorbate or EDTA was added to the volumetric flasks and larger volumetric flasks were used to further dilute the homogenates in order to obtain the sample residual results into the range of the standard curve. Results were then corrected by the proper factor.

Colorimetric Determination Of EDTA Salts In Whitefish Fillets

The method of analysis used was the modified

Darby method (1952), adapted for use in fish by Sinclair and Power (1968). EDTA preferentially complexes with nickel (added in the form of nickel sulphate), displacing all other metallic ions which are complexed with EDTA. Dimethylglyoxime precipitates out the uncomplexed nickel and the remaining nickel-EDTA chelate dissociates in acid solution, liberating the nickel which is colorimetrically determined using dithiooxalic acid solution.

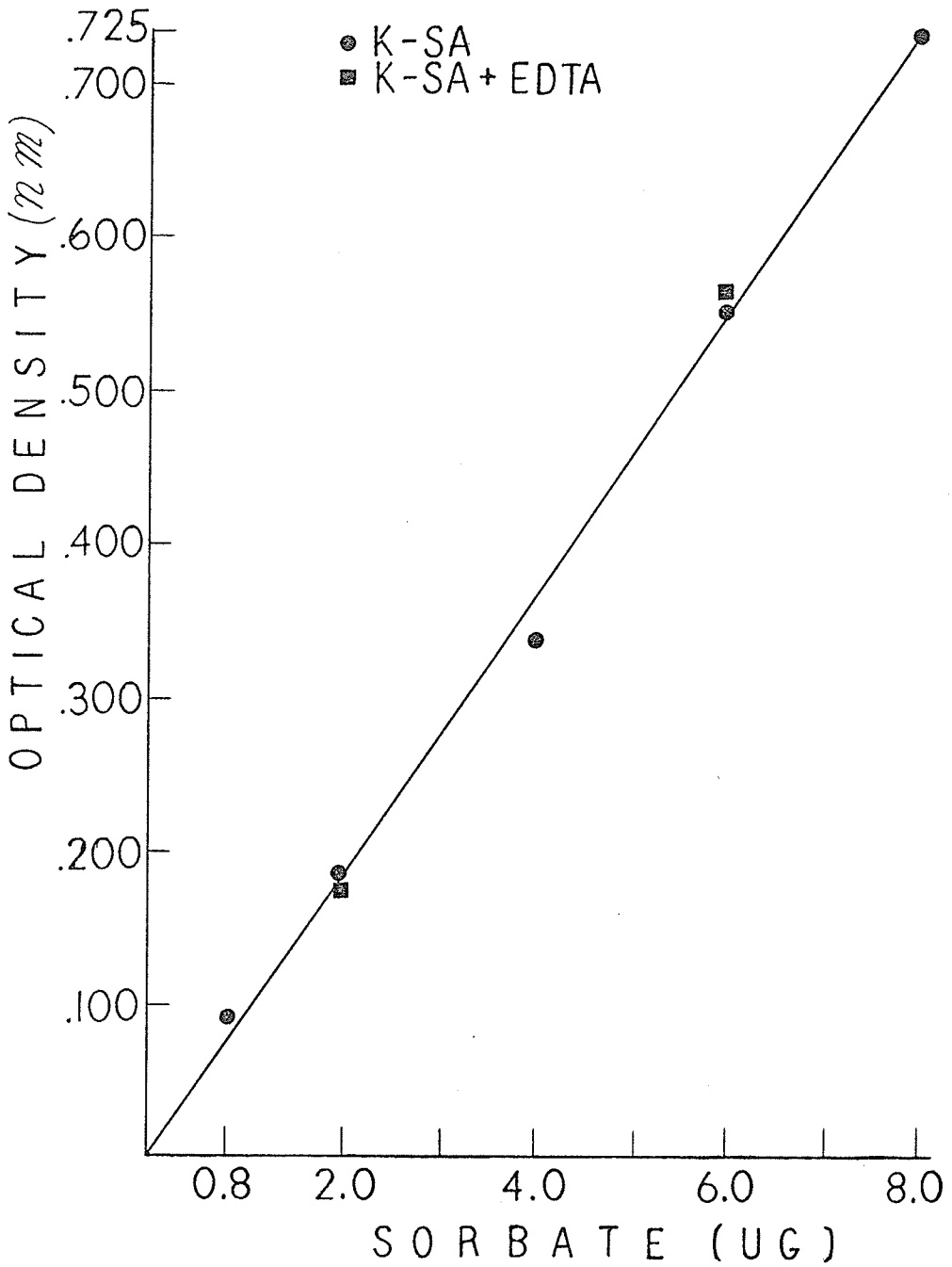
Reagents required

The trichloroacetic acid (B.D.H.) was made up to 6.3% w/v concentration by adding 6.3 gm to a 1000 ml volumetric flask and making up to volume with distilled water. The nickel sulphate solution (B.D.H.) was made up by adding 13.3 gm $NiSO_4.6H_2O$ to a one liter volumetric flask and making up to volume with distilled water. The ammonium hydroxide and hydrochloric acid (McArthur) were 28% and 35.5% respectively. The potassium dithiooxalate (Fisher) solution was made up by adding 0.1 gm of the dry chemical to a 100 ml volumetric flask and it was made up to volume not any sooner than five minutes prior to use.

Standard curve determination

Standard EDTA solution (2 mg/ml) was made up (2.562 mg Na_2 EDTA$\cdot 2H_2O$/ ml) with distilled water and added in aliquots of 0 mg (0 ml), 20 mg (10 ml), 40 mg (20 ml), 60 mg (30 ml), 80 mg (40 ml), and 100 mg (50 ml) to

FIGURE 5 STANDARD CURVE FOR POTASSIUM SORBATE IN FISH HOMOGENATE



each of six 16 ounce Canadian Mason Jars containing 100 gm of Whitefish fillet. Trichloroacetic acid was added (320 ml) and the contents blended for 3 to 5 minutes.

Duplicates of the 40 and 80 mg EDTA spiked samples were made up and equal amounts of sorbate (twice the normal concentration as before) were added to each of the two jars. These samples were used to establish whether sorbate interfered with the EDTA analysis.

Precipitated protein was then removed by filtering through Whatman #5 filter paper using suction. The next step was colour development.

Color development

Fifty ml aliquots of the TCA extracts were put into 250 ml erlenmeyer flasks. As a blank 16 ml TCA was diluted to 50 ml with distilled water. Fifteen ml of the nickel sulfate solution was added to the 250 ml erleymeyers and was mixed well by shaking. The mixture was allowed to stand for ten minutes then 5 ml of the ammonium hydroxide was added in a fume hood. The color was allowed to change from green to blue (basic pH), it was mixed well by shaking and allowed to stand for ten minutes. Next 15 ml of dimethylglyoxime solution was slowly added down the side of the flask and mixed well by shaking and it was allowed to stand for ten minutes. The decolorization step described in the Sinclair and Power (1968) method was eliminated as it removed too much colour and made colour development in

the next step impossible.

The contents of the flasks were then filtered through Whatman #40 fluted filter paper into 100 ml graduated cylinders. Twenty-five ml of the filtrate was poured into 125 ml Erlenmeyer flasks and 2.5 ml of concentrated hydrochloric acid was slowly added down the side of the flasks while gently swirling the contents. (This is twice the recommended amount of acid as the addition of only 1.25 ml of acid did not develop colour sufficiently.) The flasks were then allowed to stand for at least five minutes before proceeding any further.

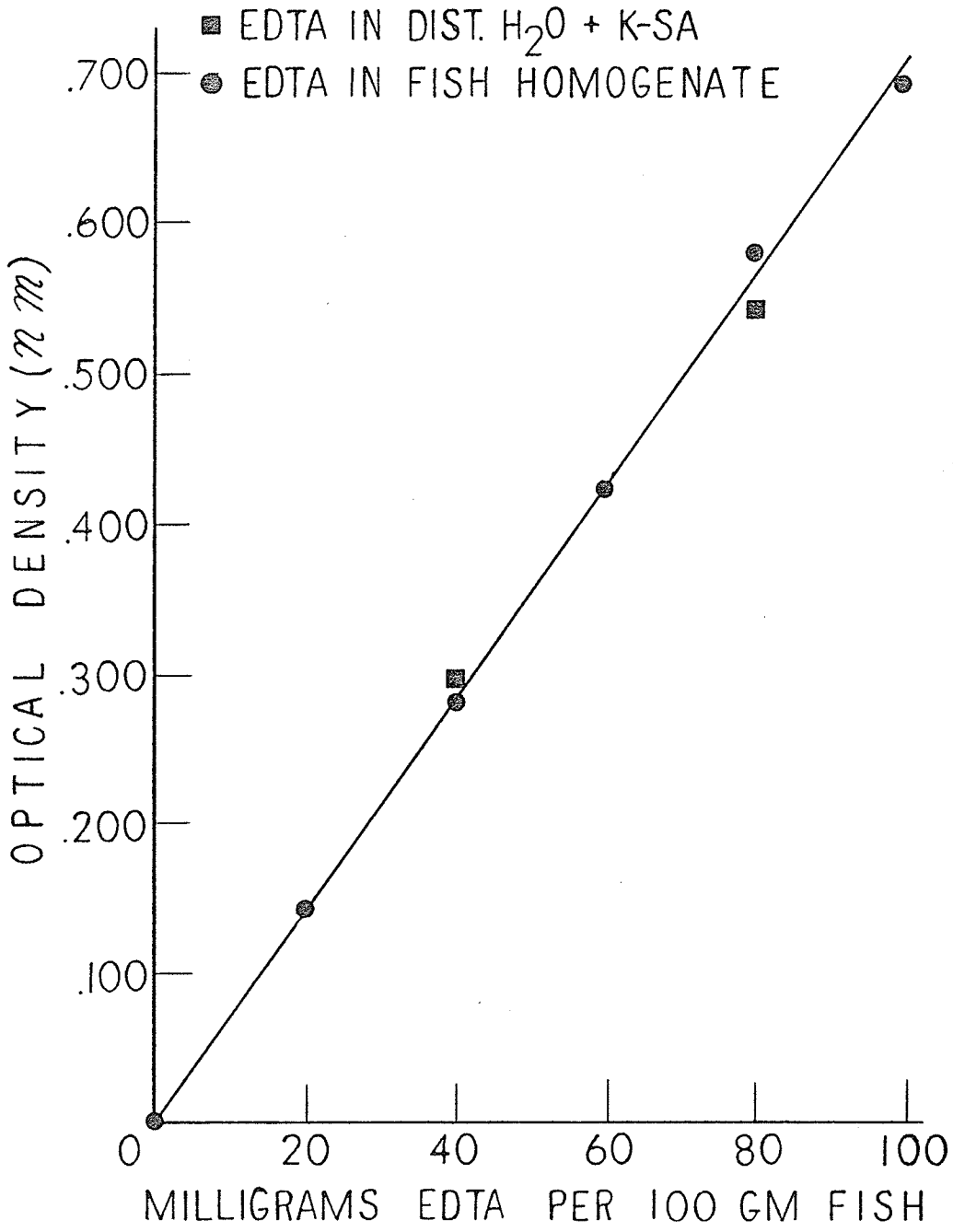
Potassium dithiooxalate solution was prepared just prior to use and 5 ml of it was added to each sample, one at a time, and read immediately for absorbance in a Spectronic '20' spectrophotometer at 510 nm. Sinclair and Power used 502 nm. This researcher found maximum absorbtion to be closer to 510 nm.

The standard curve obtained for EDTA sample analysis is shown in Figure 6.

Sample results

The same procedure as described above was used except that no EDTA was added to the samples in the blender jars. Using the standard curve for EDTA the number of milligrams of EDTA in the samples was determined and the p.p.m. residual EDTA was obtained by multiplying the milligrams of

FIGURE 6 STANDARD CURVE FOR EDTA



EDTA per 100 gm of fish by a factor of ten.

STATISTICAL ANALYSIS

Statistical Analysis Of Microbiological Data

T-test for the comparison of two samples

This test was used for the analysis of the data contained in Appendix Tables 7 to 14. Standard t-test tables were used to obtain critical values for the acceptance rejection analysis (Mendelhall, 1971).

Completely randomized design (one-way analysis of variance)

This testing procedure was applied to the data in Appendix Tables 15 to 32. The ANOVA tables for these analyses are to be found in Appendix Tables 34 to 43. The critical values for the Fisher distribution were obtained from standard F-test tables (Mendelhall, 1971).

Statistical Analysis Of Organoleptic Evaluation Data

Wilcoxon signed ranks test for paired samples

This test was used for the analysis of the odour scores data contained in Appendix Tables 44 to 56. A table for quantiles of this test statistic was used to determine the significance of the results (Conover, 1971).

Cox and Stuart test for trend

This test was used to analyze the taste panels results for aroma and taste. These results are tabulated in Tables 5 and 7. The analytical method for this test was derived from Conover (1971).

The taste panel results for general appearance and texture were not statistically analyzed. A colour testing apparatus such as an Agtron meter would have to have been used to accurately examine appearance, and a texturometer would have been required to accurately examine texture. Since it was not intended to examine these characteristics in such depth the results were only visually examined for obvious trends and the results were reported in the 'results' section. The results are tabulated in Tables 9 and 10.

The order in which the various experiments were done is the order in which they appear in the results section. Odour investigations were carried out on the samples pieces before they were subjected to microbiological analysis. The taste panels were conducted as an entirely independent study because of the sheer numbers of samples required to be analyzed. The number of duplicates, replicates, and judges involved in these experiments are indicated in the Appendix Tables for the respective experiments. Chemical examinations were done as an independent study after the taste panel samples were treated and stored.

RESULTS

PRELIMINARY MICROBIOLOGICAL EXAMINATIONS

Preliminary investigations into the use of sorbic acid on Whitefish fillet pieces showed that concentrations of from 0.05 to 3.0% w/v potassium sorbate (Appendix Tables 1 to 4) had little or no significant effects upon bacterial numbers initially and no effect upon their subsequent growth at a storage temperature of $3^{o}C$. However, the suppression of yeasts and molds was quite evident. It should be noted that small consistent differences existed between controls and treatment counts for concentrations as low as 0.25% w/v sorbic acid and shelf life extensions could be approximated at almost one day over the controls. It had been determined that at the approximate level of 10^{8} viable psychrotrophic organisms per gram of fish the controls showed the first definite signs of spoilage which was determined by odour evaluation (Table 3, pp. 50). The treatments however, did not usually reach this level at least until some time later. This gap between the two times required to reach the 10^{8} level was arbitrarily chosen as the shelf life extension value.

The 0.50, 0.70, and 1.00% w/v sorbic acid treatments showed shelf life extensions of just over one day (Appendix Tables 2, 3, 4, and 7). At the 3% w/v level a shelf life extension of approximately 2 days was observed

(Figure 7), and at the 5% w/v level shelf life extensions of 4.5 to 5.5 days were observed (Figure 8).

Very poor results were shown for the calcium disodium salt of EDTA (Appendix Tables 5 & 6). After initial testing of calcium disodium EDTA on fillet pieces it was determined that no significant effects were demonstrated as high as the 4% w/v 5 minute exposure time treatment level. It was therefore decided to discontinue further testing of the calcium disodium salt and to concentrate on the disodium salt of EDTA

PRELIMINARY ORGANOLEPTIC EVALUATIONS

Odour evaluations revealed differences in the effects of the same treatment on bacterial growth and the formation of odour. In general, the development of odour was delayed for a longer time than the bacterial counts, to reach the same level as the controls. In various treatments it was observed that for example, a treatment could have reached the 10^8 level one day later than the controls; but the odour evaluation was not the same or at least was not unacceptable until two or three days later. At the 0.25% w/v treatment level, after five days the controls began to smell sour whereas it was not until after six or seven days before the treated samples began to smell. Similarly, the 0.50, 0.70, and 1.00% w/v treatments showed extensions of two to four days over controls.

FIGURE 7 THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS

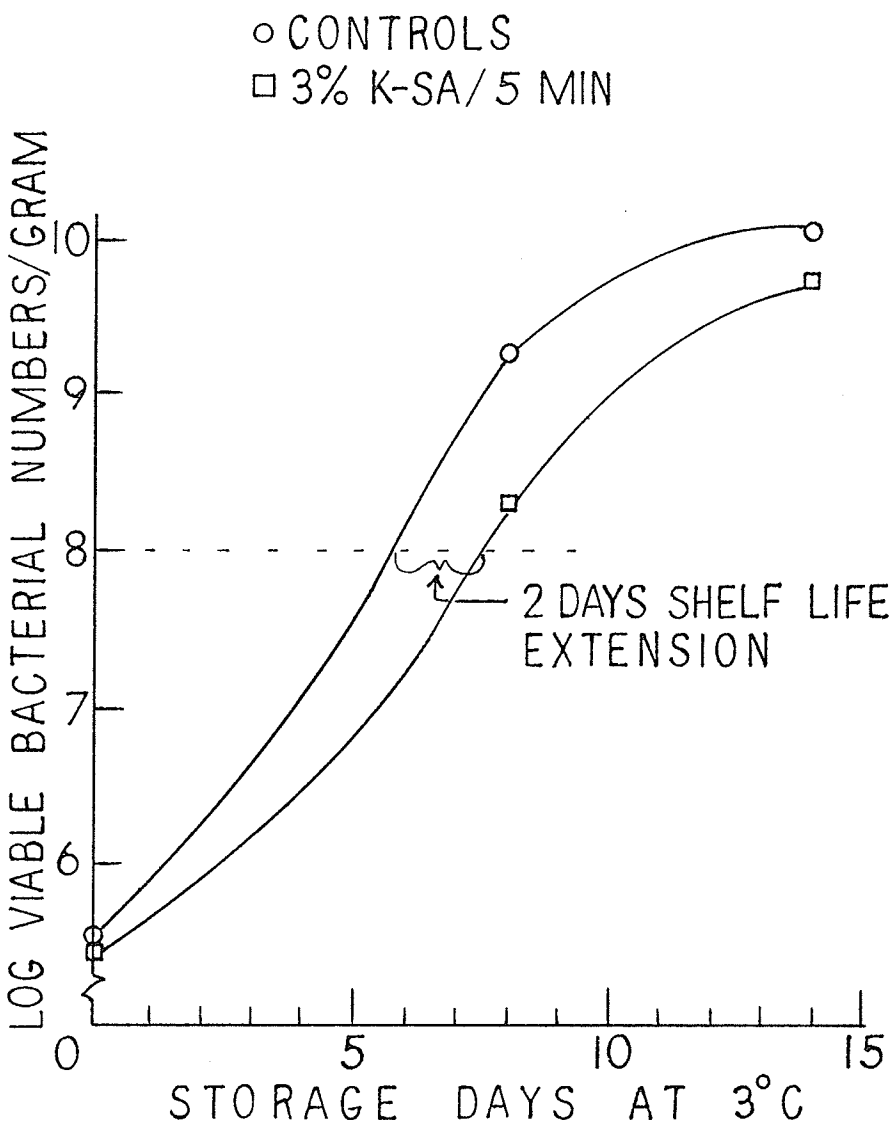
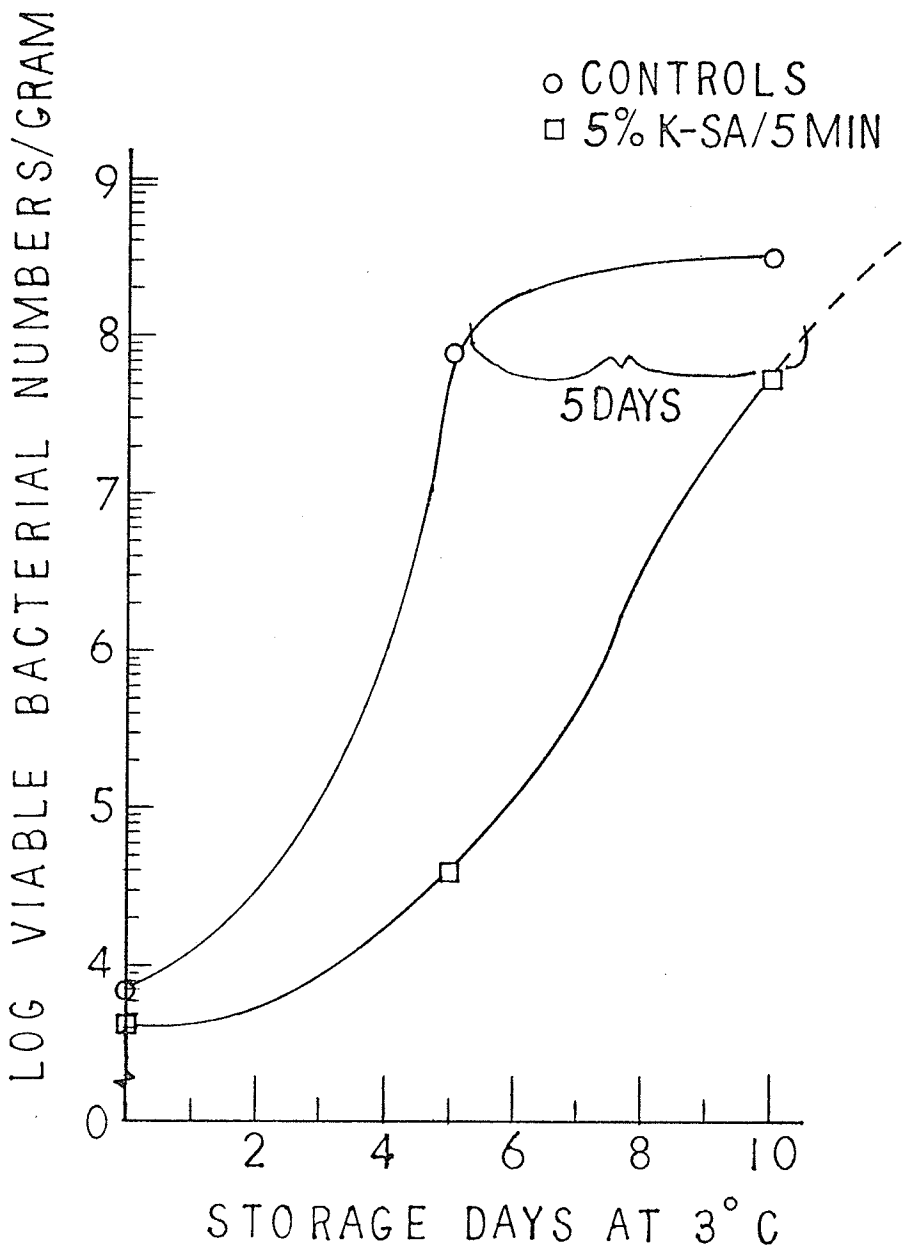


FIGURE 8 THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS



MICROBIOLOGICAL EXAMINATIONS

Fillet Pieces Treated With Potassium Sorbate

In general, it was observed that the use of potassium sorbate solutions in extending the shelf life of fresh Whitefish fillet pieces seemed to be successful in forestalling odour and rancidity development due to bacterial growth. In lower concentrations of 4.0 to 6.0% w/v potassium sorbate the growth of bacteria was increasingly inhibited as treatment concentration was increased (Figures 9, 10, and 11). In all three cases (4.0, 5.0, and 6.0% w/v potassium sorbate) there was observed a noticeable difference in viable counts for the treatments as compared with the controls. At the 4% potassium sorbate level bacterial counts on the treatments were lower than control counts (significant to over the 95% confidence interval). Initial counts for the treatments showed a decrease of 1.4 log cycles from the control level. At five days storage time treatment counts were lower than controls by approximately 1.65 log cycles and at ten days by approximately one-half log cycle. The estimated shelf life extension obtained by a five minute exposure to 4% w/v potassium sorbate was about 3.5 days. Increased effects were observed with the 5% w/v potassium sorbate (five minutes exposure time) treatment. At ten days there was a difference of approximately 1.9 log cycles between treatments and controls. Shelf life extension was estimated to be about 5.5 days (Table 4, p.71).

FIGURE 9 THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS

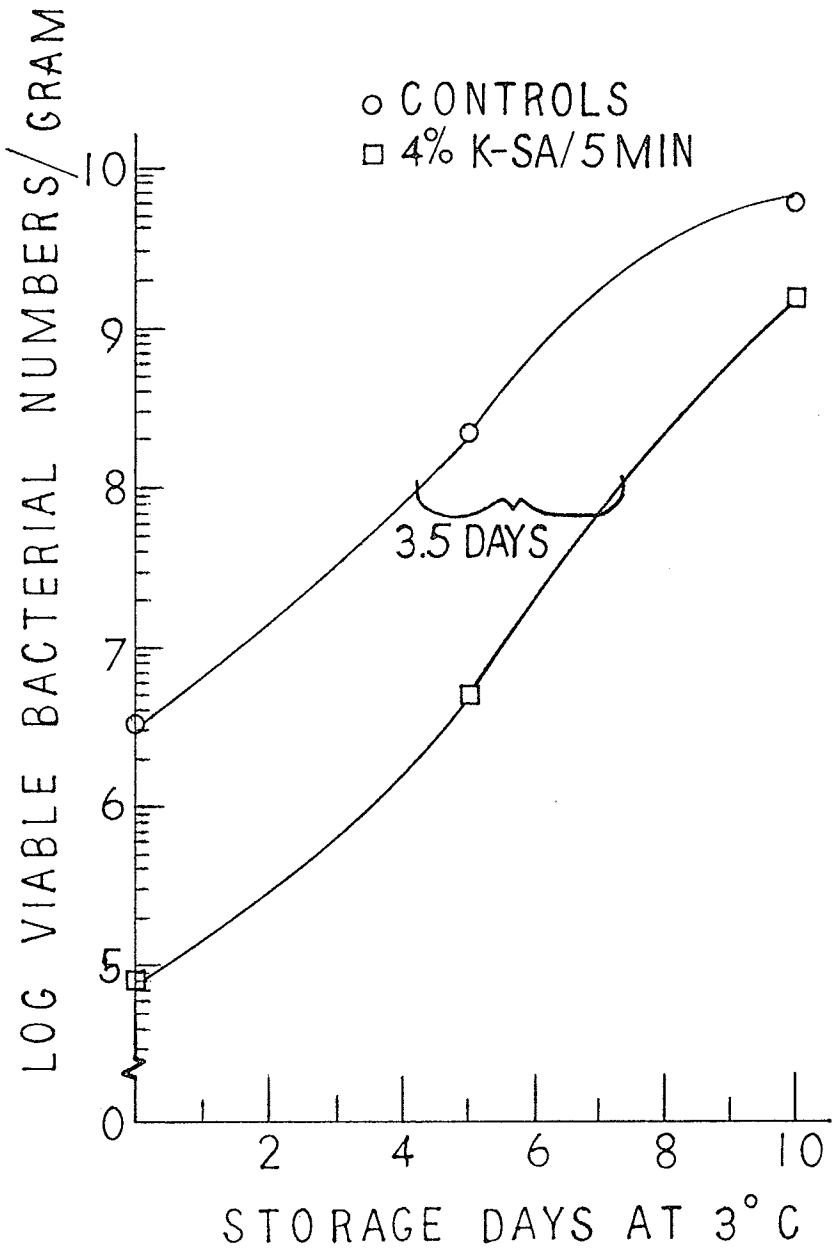


TABLE 4

THE EFFECT OF POTASSIUM SORBATE AND EDTA TREATMENTS

ON SHELF LIFE EXTENSION OF FRESH WHITEFISH FILLETS

TREATMENT CONCENTRATION	POTASSIUM SORBATE	DISODIUM EDTA
0.05%	less than one day	not available
0.10%		
0.25%		
0.50%	1.0 to 1.5 days	not available
0.70%		
1.00%		
3.00%	2.0 days	not available
4.00%	3.0 to 3.5 days	2.0 days
5.00%	5.5 to 6.0 days	not available

FIGURE 10 THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS

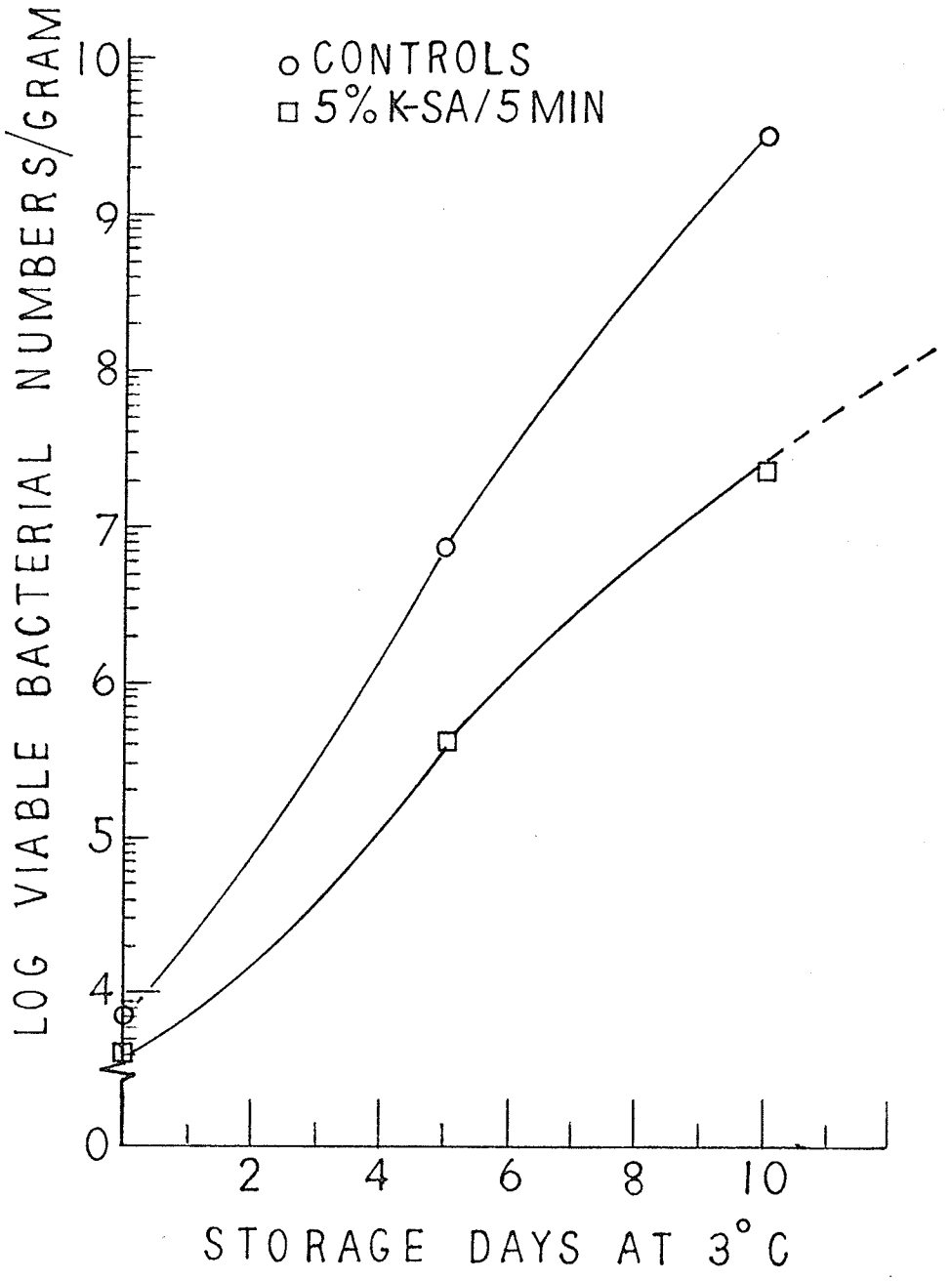
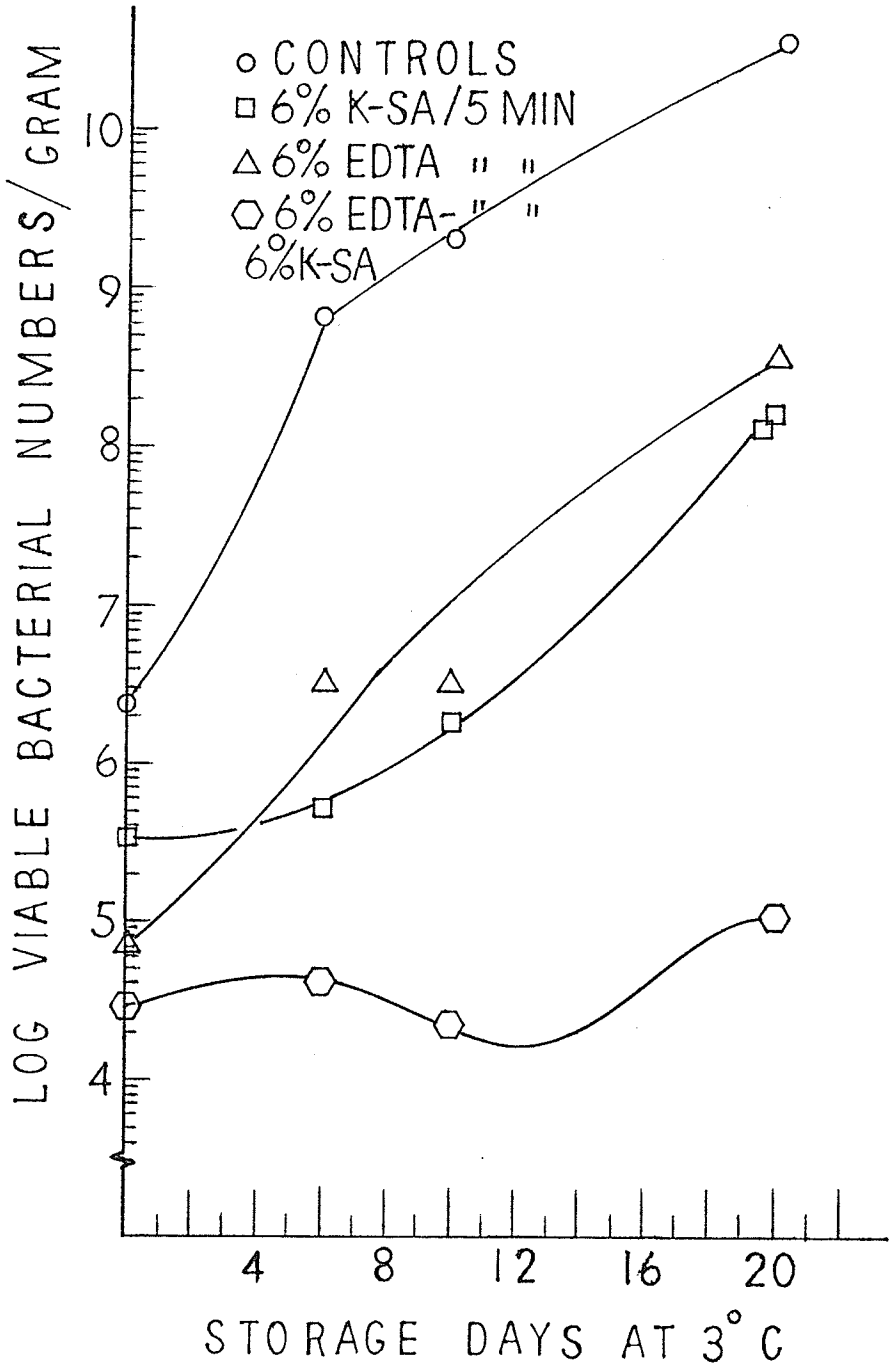


FIGURE II THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS



At roughly the 6% w/v treatment concentration level even more significant decreases in initial counts were noticed, along with greatly increased differences in counts at each analysis time. At six days post treatment there was a difference of 3.2 log cycles, at ten days 3.0 log cycles, and at twenty days 2.2 log cycles. Shelf life extension at the 10^8 level was estimated to be about fifteen days.

The 8% w/v potassium sorbate treatments revealed an initial drop in counts of approximately 1.1 log cycles as shown in Figure 12 and a further drop at six days storage time with a difference in counts between treatments and controls of approximately 3.7 log cycles. There was some recovery by ten days when the count was approximately 1.5×10^5 cells/gm, but the difference between that and the controls was about 4 log cycles. At twenty days the difference was about 4.7 log cycles. The shelf life extension could not be calculated; however, it could be estimated at over twenty days.

When different exposure times were compared using the 8% w/v potassium sorbate as the treatment concentration (Figure 13) it was shown that three and five minutes exposure time effected similar results whereas the seven minutes exposure time treatment only showed similar results up to six days. After six days counts were all significantly lower than for the three and five minutes exposure time treatments.

FIGURE 12 THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS

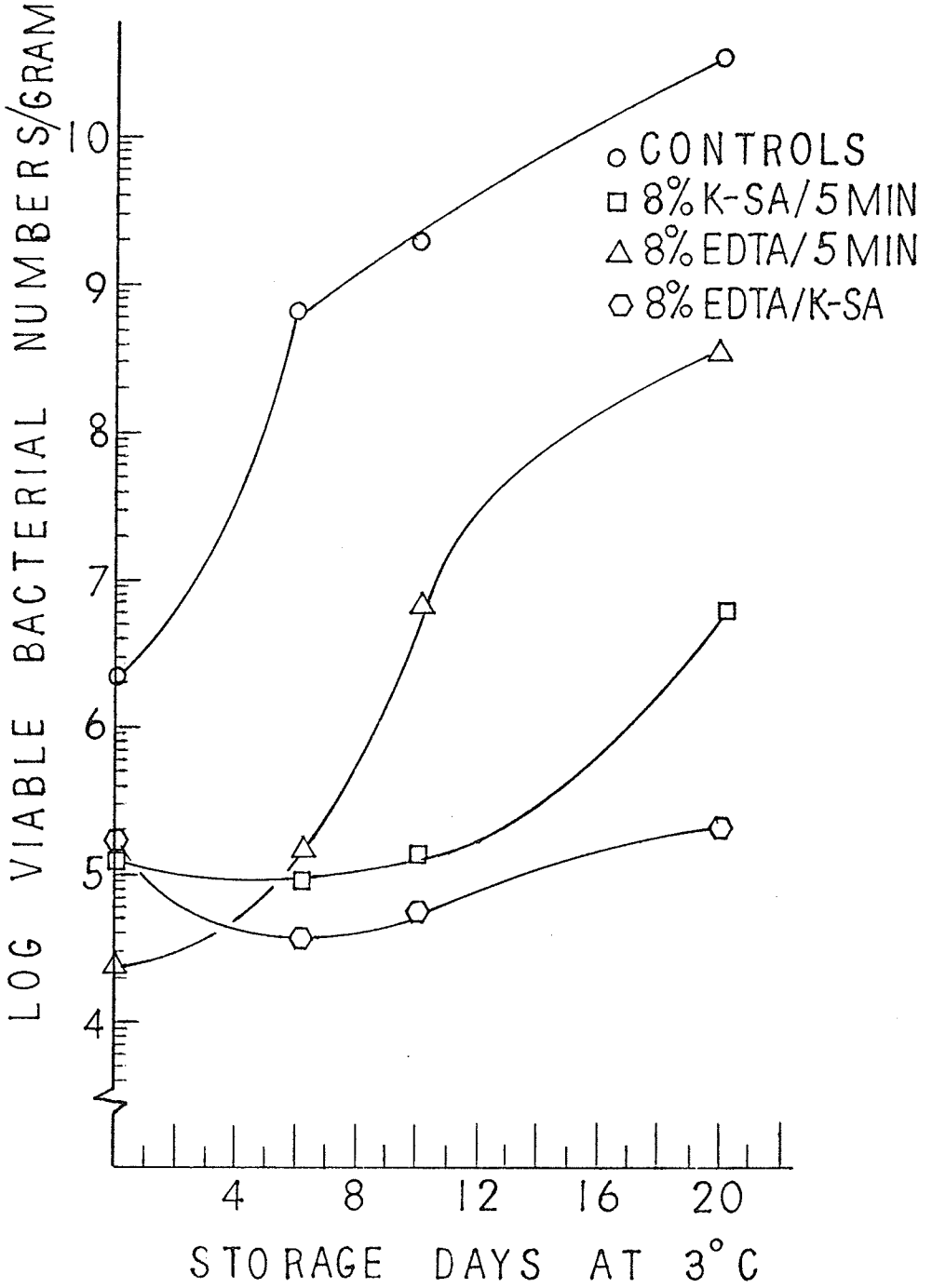
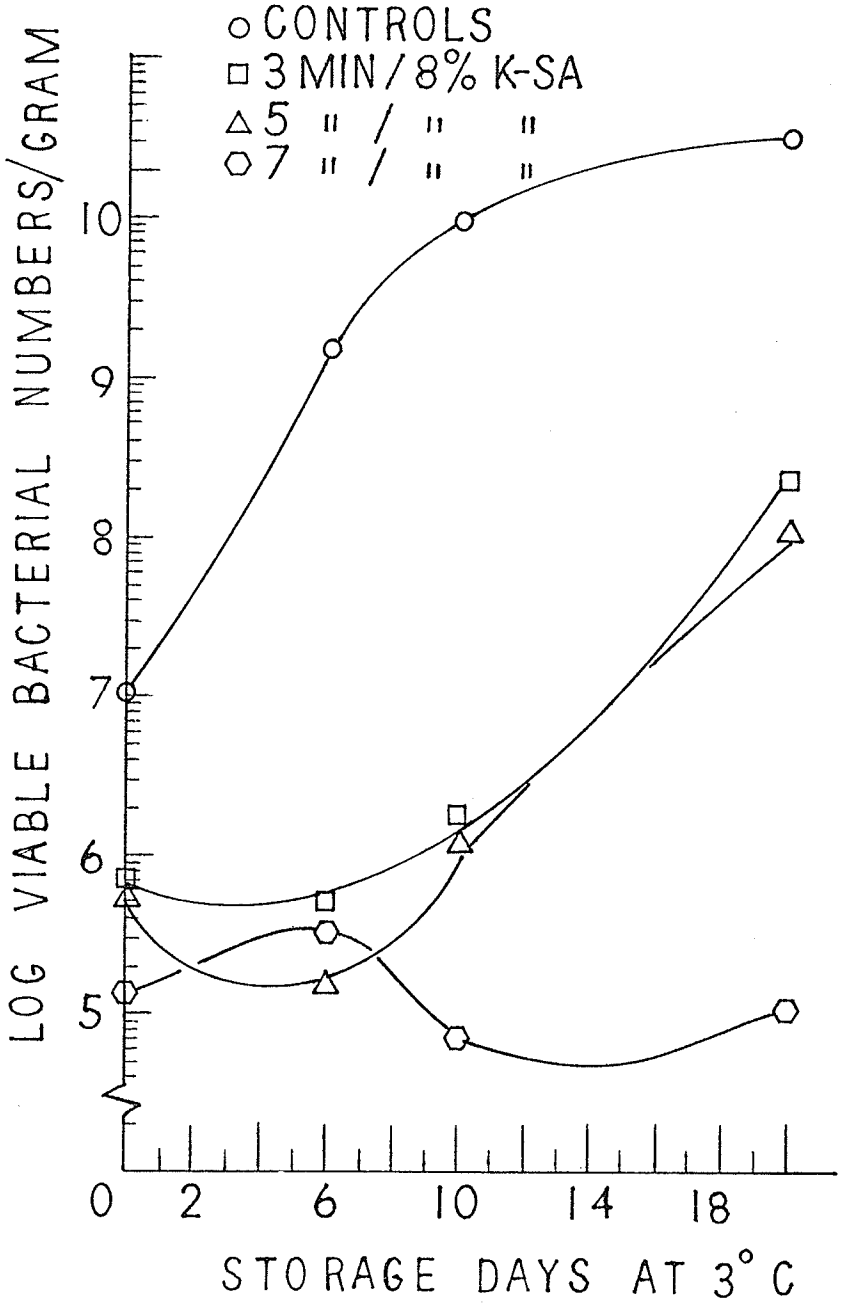


FIGURE 13 THE EFFECT OF POTASSIUM SORBATE AT VARYING EXPOSURE TIMES ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS



All treatment counts were significantly lower initially than the controls and log cycle difference values were 1.3, 1.5, and 2.0 for the 3, 5, and 7 minutes treatments, respectively. All treatments showed declines in viable numbers up to ten days storage time and all increased after ten days. Shelf life extensions of approximately 16.0 and 17.5 days were observed in the three and five minutes exposure times, respectively. The extension for the seven minute treatment could not be estimated but it was probably over 20 days.

The 9% w/v potassium sorbate treatment showed no significant initial decrease in counts at zero time. However, between zero and ten days storage time marked declines in numbers and marked differences between treatment and control counts were observed. The shelf life extension statistic could not accurately be determined at the 10^8 level as that sample was contaminated before analysis time. Figure 14 shows what the growth curve would probably have looked like.

The 10% w/v potassium sorbate treatment had drastic effects on bacterial numbers as levels stayed about constant with the initial counts as shown in Figure 15. At ten days bacterial counts were four log cycles greater in the controls than for the treated samples. Although no shelf life extension could be calculated, it was bound to be in excess of twenty-five days.

FIGURE 14 THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS

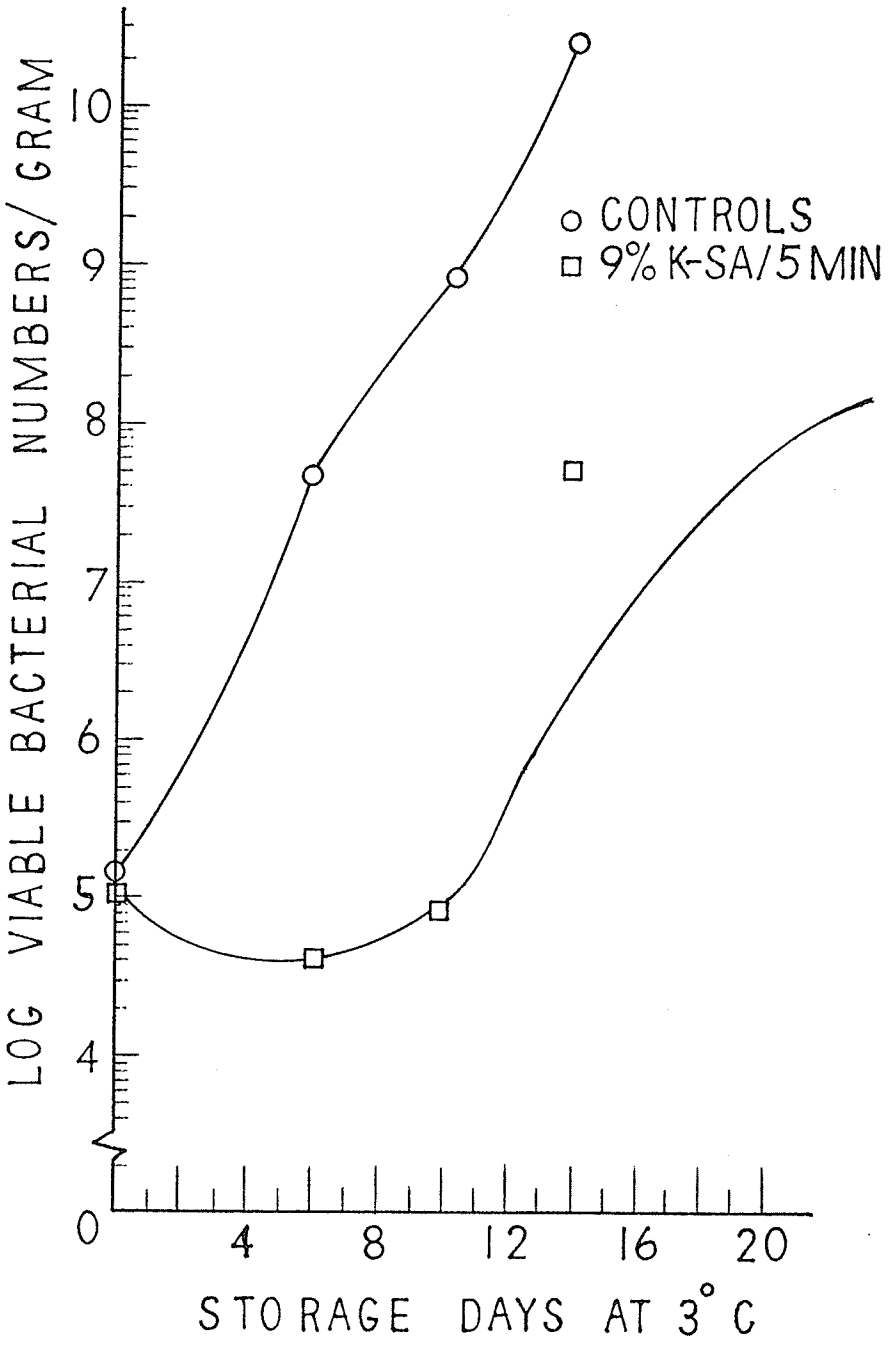
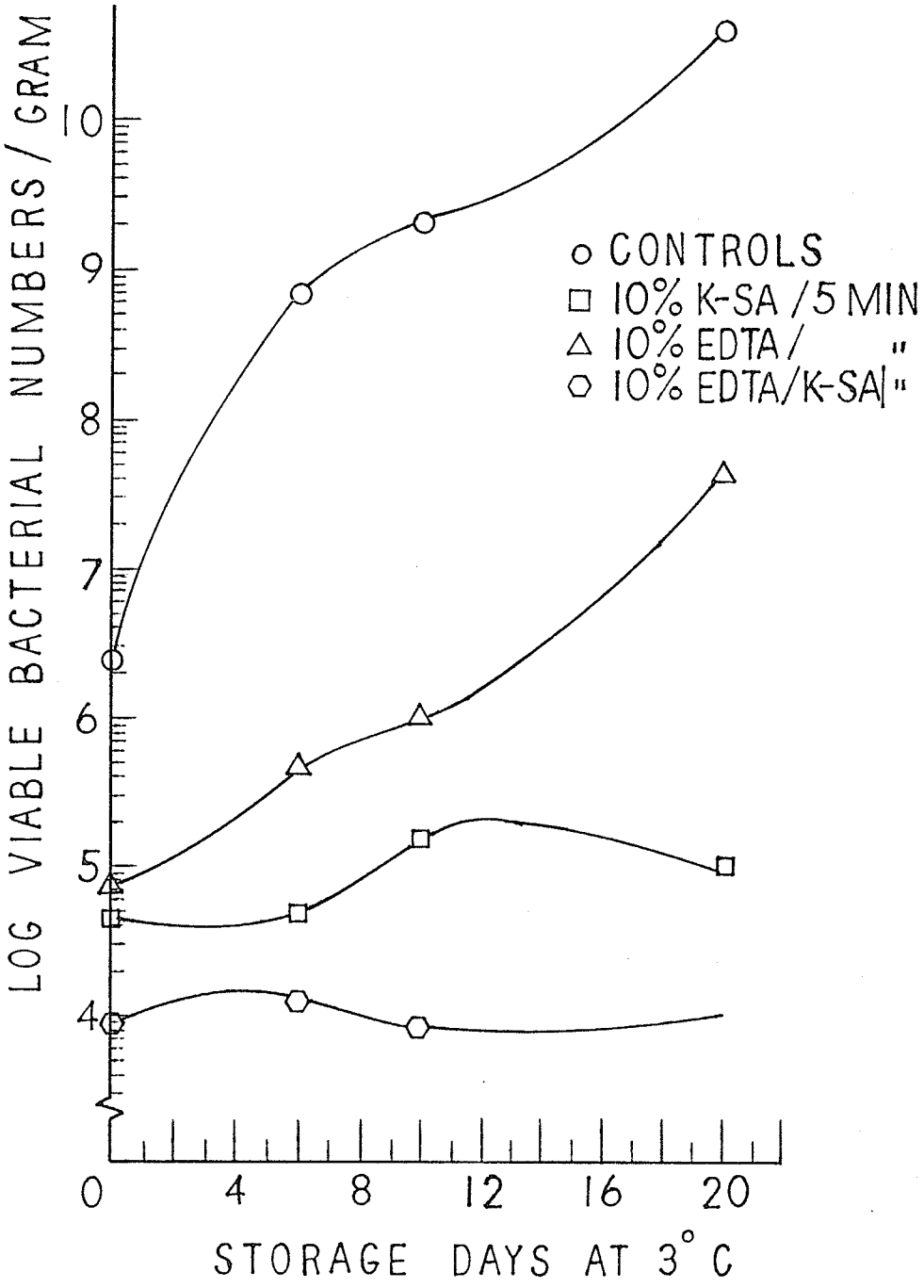


FIGURE 15 THE EFFECT OF POTASSIUM SORBATE DISODIUM EDTA AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS



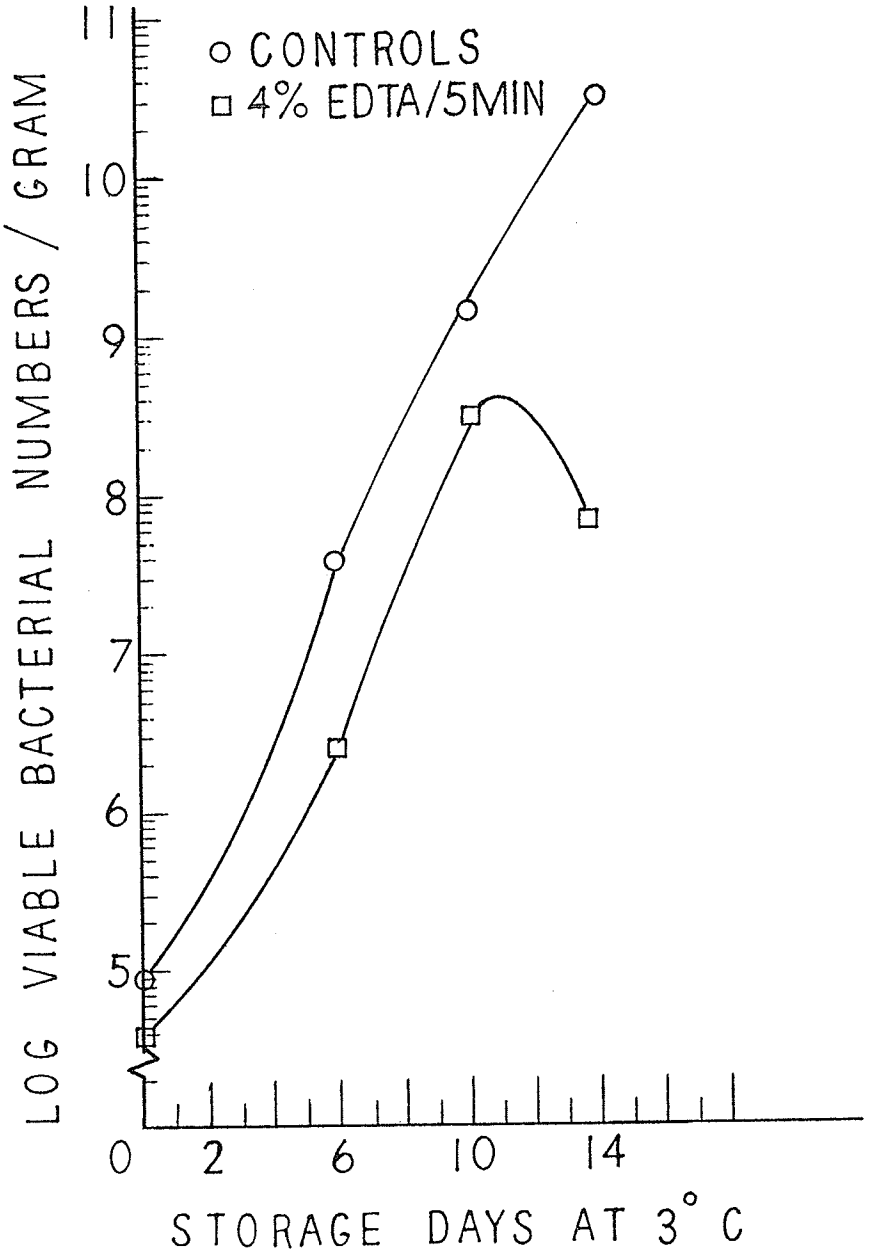
Fillet Pieces Treated With Disodium EDTA

The same treatment concentration and exposure time for $EDTANa_2$ did not have the same effects on bacterial numbers as did the potassium sorbate treatments. The results for the 4% w/v disodium EDTA treatments showed an initial decrease of 0.50 log cycles from the control level, whereas the initial decrease for the sorbate treatment was 1.4 log cycles. At five days storage time counts on the 4% disodium EDTA treated samples lagged behind controls by only one log cycle as compared with 1.65 log cycles for the same concentration and exposure time for potassium sorbate. At ten days the lag was 0.75 log cycles as compared with 0.50 log cycles for the sorbate treatment. However, the shelf life extension at the 10^8 level was only two days as compared with 3.5 days for the sorbate treatment (Figure 16).

The 6% w/v disodium EDTA treatments showed markedly increased effects on bacterial growth (Figure 11). There was an initial decrease of 1.3 log cycles as compared with the controls. At five days the difference was 2.1 log cycles below the control values, at ten days 2.75 log cycles below controls, and finally at twenty days 2.0 log cycles below controls. Shelf life extension was determined to be 13.5 days as compared with 15 days for the 6% w/v potassium sorbate treatments.

The 8% w/v disodium EDTA treatments showed little

FIGURE 16 THE EFFECT OF DISODIUM EDTA ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS



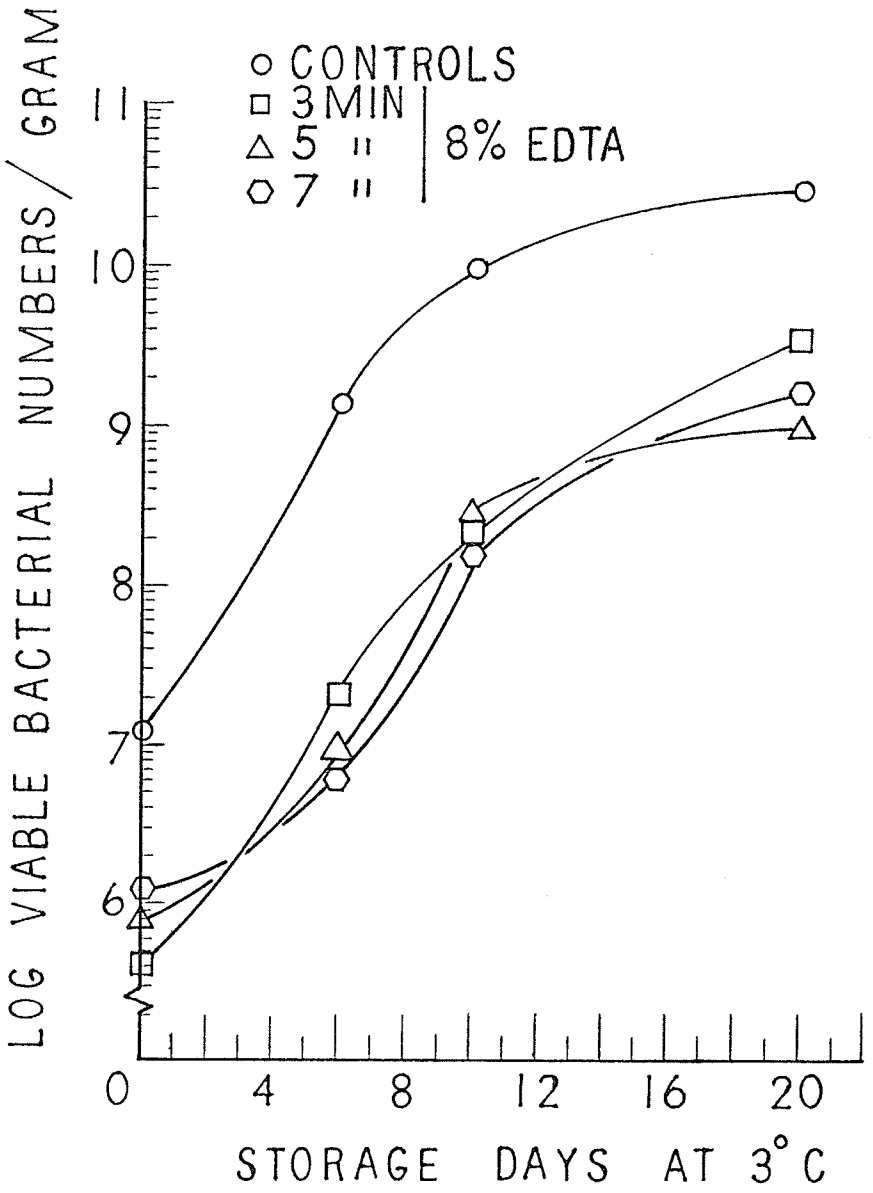
difference for varying exposure times (Figure 17). All treatments showed initial decreases at the zero time analysis of about one log cycle below controls as compared with approximately 1.5 log cycles for the sorbate treatments of the same concentration and exposure time. Figure 12 indicates a shelf life extension of approximately 13 days at the 10^8 level when compared with controls as compared with over twenty days for the 8% w/v sorbate treatments. At five days storage time, the counts for the 8% EDTA treatments lagged behind controls by about 3.2 log cycles as compared with 3.3 log cycles for the sorbate treatments. At ten days the lag was approximately 2.4 log cycles as compared with about 4.1 log cycles for the sorbate treatments.

The 10% disodium EDTA treatments showed markedly increased effects on bacterial numbers as compared with the 6 and 8% w/v disodium EDTA treatments (Figure 15). At five days the EDTA treatment counts were three log cycles below that of the controls and at ten days the difference was 3.1 log cycles, while at twenty days the difference was 3.0 log cycles once again. Shelf life extension was determined to be approximately nineteen days.

Fillet Pieces Treated With A Combination Of Potassium Sorbate and Disodium EDTA

The most significant effects on bacterial numbers were observed with the fillet pieces treated with the combin-

FIGURE 17 THE EFFECT OF DISODIUM EDTA AT VARYING EXPOSURE TIMES ON THE VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS



ation treatment of disodium EDTA followed by potassium sorbate. An initial decrease of 1.9 log cycles below controls was observed for the 6% level of combination treatment as compared with 1.35 and 0.9 log cycles for the 6% disodium EDTA and 6% potassium sorbate treatments, respectively (Figure 11). At five days storage time a similar pattern of lags was observed. The combination treatment counts lagged behind controls by 4.2 log cycles, and the EDTA and sorbate treatments lagged behind by 2.4 and 3.3 log cycles, respectively. At ten and twenty days storage time similar lag patterns were observed with the combination treatments showing the best observed results. A shelf life extension could not be reasonably determined as the bacterial counts did not exceed 10^5 organisms per gram.

The 8% combination treatments did not show results better than the EDTA and sorbate treatments at zero time but by six days storage time counts had fallen lower than the other treatments (Figure 12). At zero time counts for the combination treatments were about 1.1 log cycles lower than controls. At six days storage time counts were about 4.4 log cycles lower than controls and about 0.75 and 0.55 log cycles lower than the EDTA and sorbate treatments, respectively. For 6, 10, and 20 days storage time the combination treatments counts paralleled the sorbate treatments, being constantly about 4.5 to 5.5 log cycles lower than the sorbate

counts. Once again, a shelf life could not be derived as the combination counts did not exceed 10^6 organisms per gram.

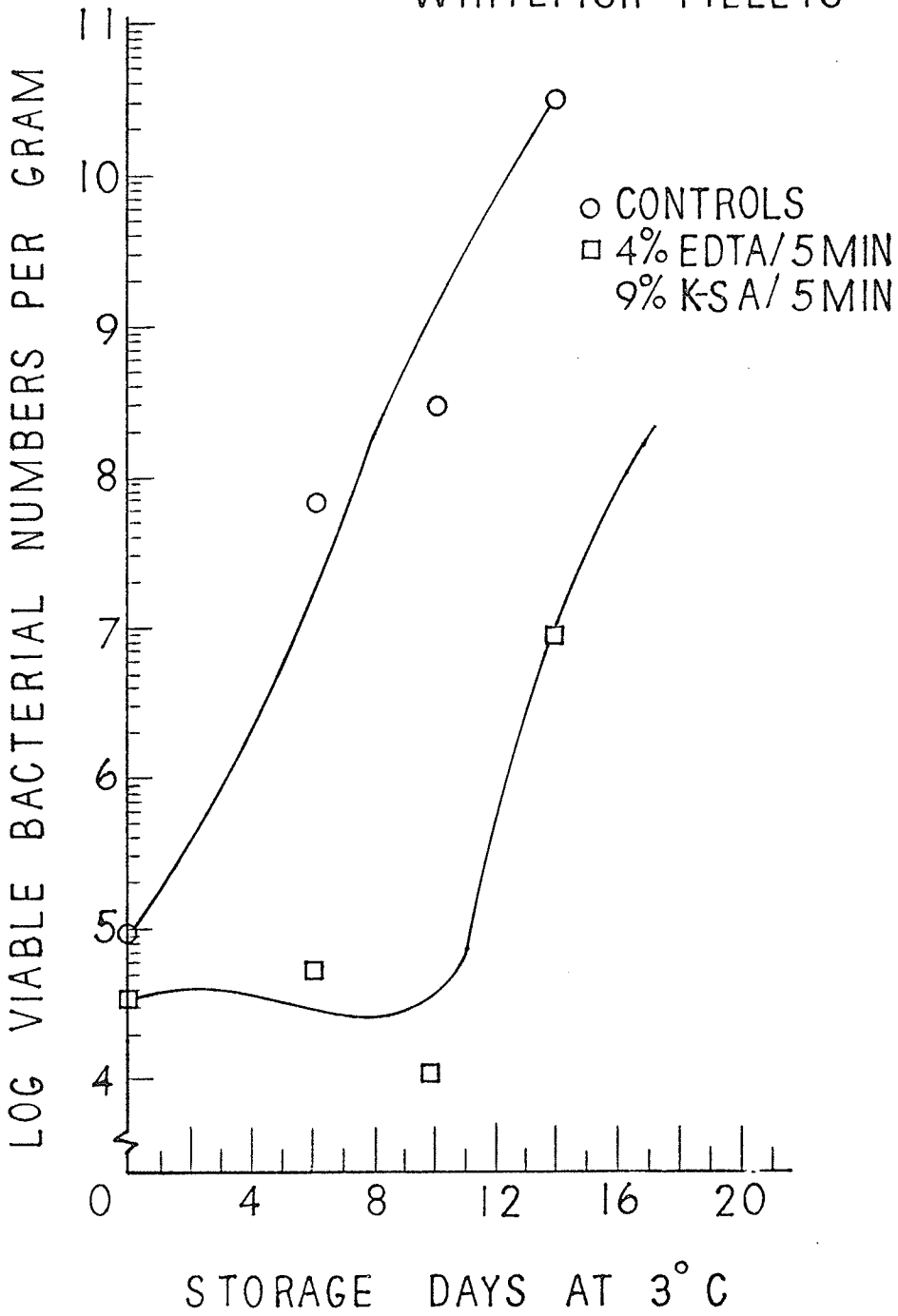
The 10% combination treatments yielded results similar to the 6 and 8% treatments up to ten days of storage (Figure 15). The analysis sample for 20 days was contaminated during the analysis procedure and an unreasonably high count resulted. The treatment and analysis were not repeated. Instead, a plot was extended by extrapolation from the ten days level taking into account the 0,6, and 10 days counts and the results for the 6 and 8% treatments for twenty days storage time.

A 4% disodium EDTA treatment combined with a 9% potassium sorbate treatment showed an initial decrease in counts from zero to ten days storage time (Figure 18). At ten days the combination treatment results were more than 4.0 log cycles lower than controls. Although the counts were radically higher at 14 days (10^7 cells/gm as compared with 10^4 cells/gm at ten days) , they were still considerably lower than controls, by about 3.2 log cycles. Shelf life extension could be roughly estimated to be about nine to ten days over controls at the 10^8 level of organisms per gram.

Studies On The Main Spoilage Flora

Results using the penicillin sensitivity test did not conclusively show that Pseudomonas spp. was responsible for the normal cold storage spoilage of the Whitefish

FIGURE 18 THE EFFECT OF POTASSIUM SORBATE IN COMBINATION WITH DISODIUM EDTA ON VIABLE PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS



fillets. The tests also did not conclusively show that any of the treatments had a significant effect on Pseudomonas organisms exclusively. The treatments were observed to have similar effects on all organisms present. It was observed that the penicillin resistant bacterial counts started out in low numbers of about 5 to 30% of the total. By 10 days storage time these counts composed about 45 to 95% of the total. By twenty days storage time they were composed of about 70 to 100% of the total (Figures 19, 20).

Trends towards differences among the treatments were closely related to plots formed by counts on the regular SPC plates. These growth/time curves followed the same general patterns laid out by those of the regular SPC counts. There was a gradual increase in Pseudomonad numbers but at a proportionately lower total numbers rate acceleration when compared with controls. This could be interpreted as specific growth inhibition of Pseudomonas.

Once again the combination treatments were the most effective. The potassium sorbate treatments were not quite as effective but still were fairly good. The EDTA treatments, although better than the potassium sorbate treatmentsments for the first 3 days, were the least effective of all treatments over the remainder of the storage period.

FIGURE 19 THE EFFECT OF POTASSIUM SORBATE AND DISODIUM EDTA AT VARYING EXPOSURE TIMES ON VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA IN WHITEFISH FILLETS

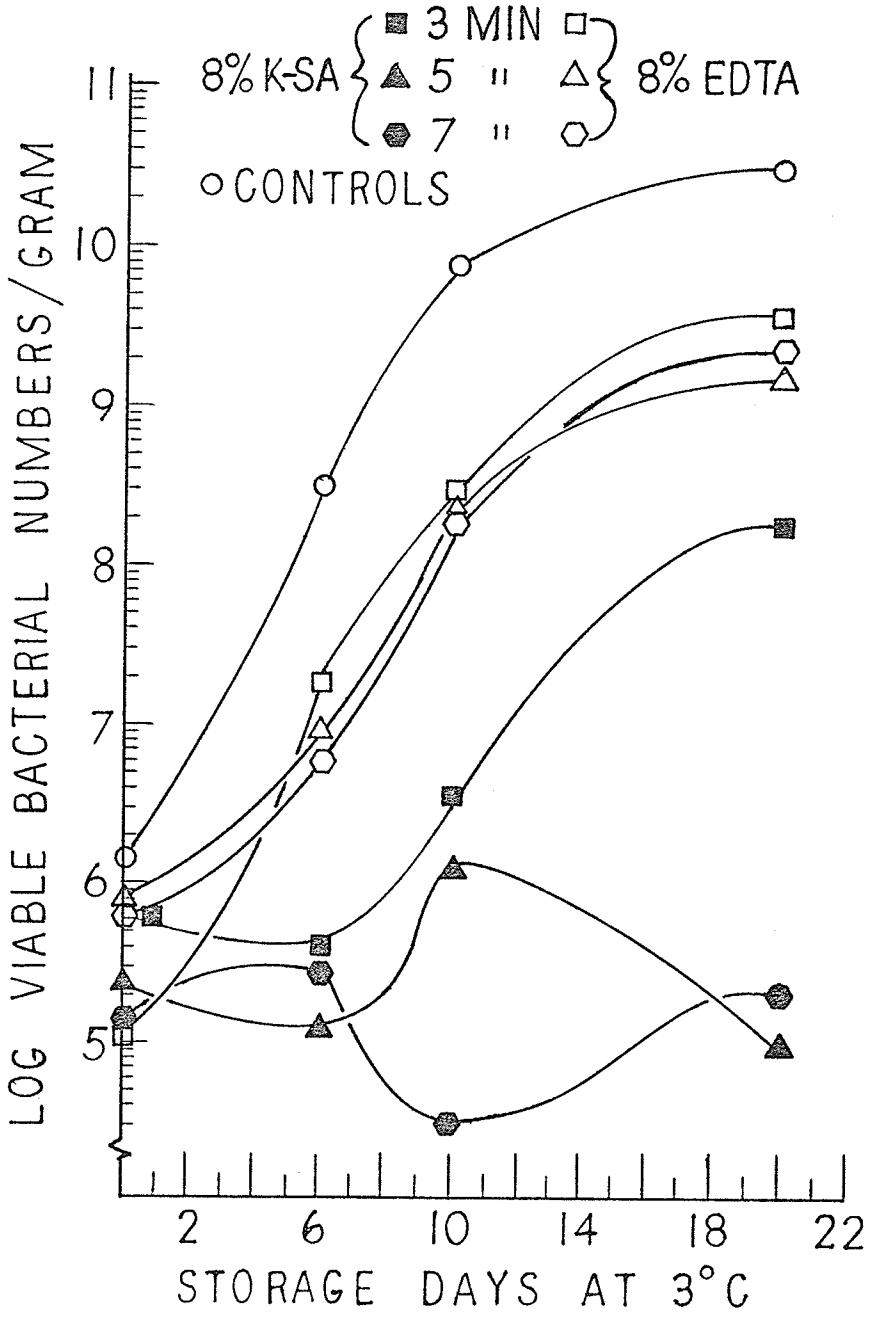
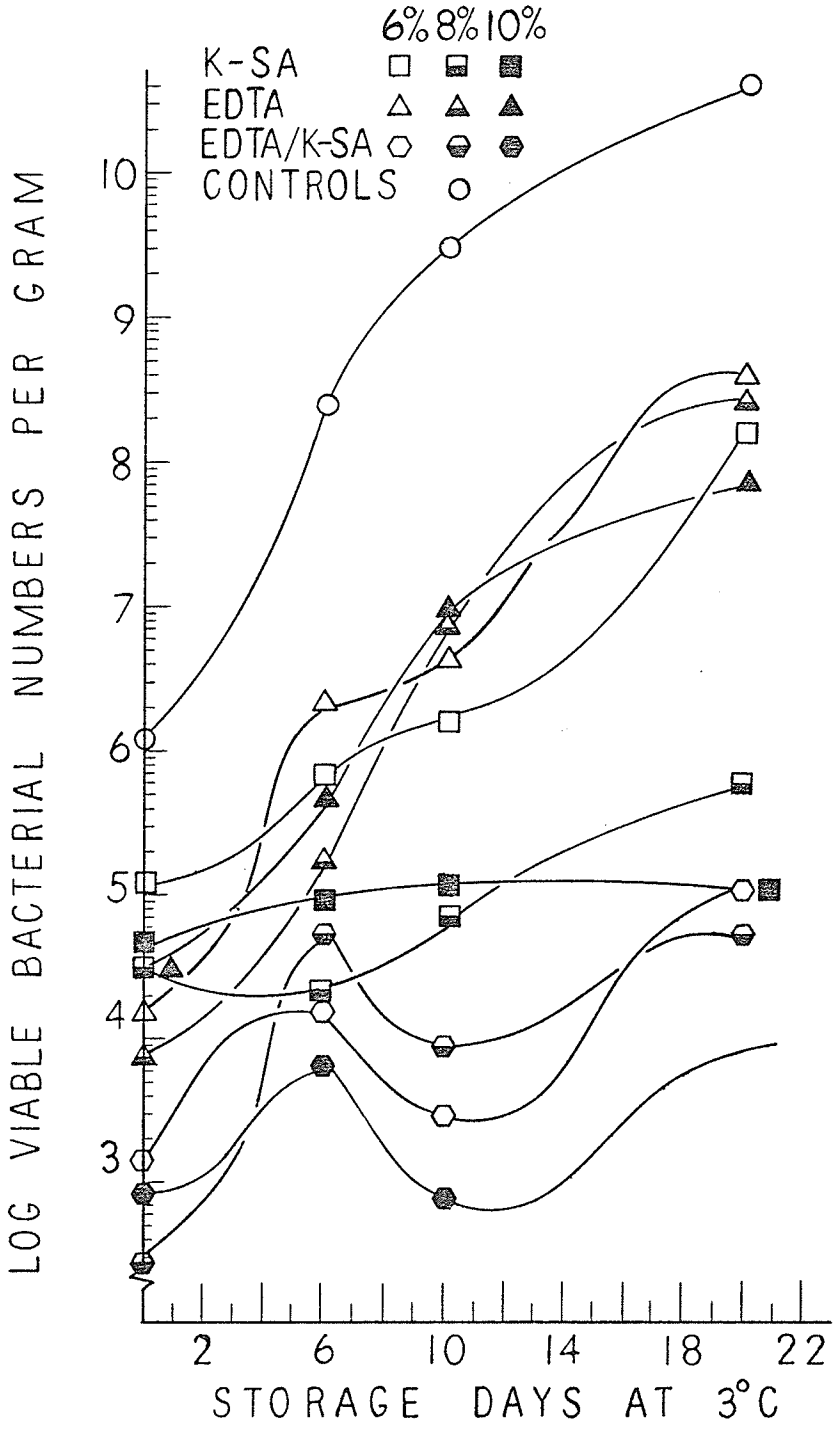


FIGURE 20 THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PENICILLIN RESISTANT PSYCROTROPHIC BACTERIA IN WHITEFISH FILLETS



SENSORY EVALUATION OF FILLET PIECES

Odour Investigations

EDTA treatments

There was a significant difference (at the 90% confidence interval) in odour ratings between controls and both the 4% disodium EDTA and 4% calcium disodium EDTA treatments (Figure 21). However, there appeared to be no significant difference between the two EDTA salts in their effectiveness in forestalling odour development.

The 6% disodium EDTA treatments were significantly better than controls, but there was no significant difference when they were compared with the 8 and 10% disodium EDTA treatments or the 6% combination and 6% potassium sorbate treatments. Comparing the 6% disodium EDTA treatments with the 8 and 10% levels of the potassium sorbate and combination treatments showed no significant difference at the 90% confidence interval; there was however, a significant difference at the 80% confidence interval for the comparison of 6% disodium EDTA with the 10% potassium sorbate and combination treatments (Appendix Tables 51 to 56).

At the 8% level of treatment, significant differences were observed between all treatments and controls but no significant differences were observed among the treatments for different exposure times (Figure 22). There were no significant differences when the 8% level was compared

FIGURE 21 THE EFFECT OF EDTA ON ODOUR RATINGS

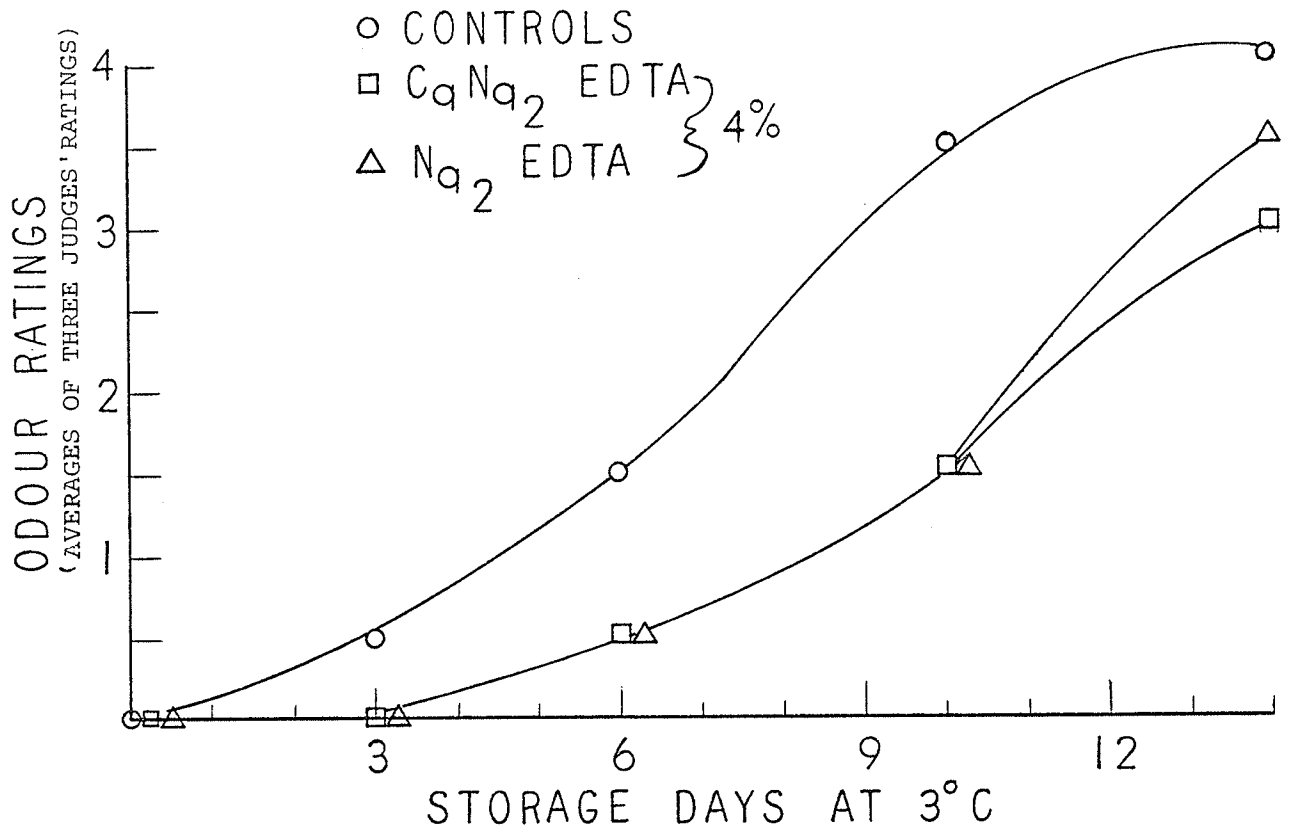
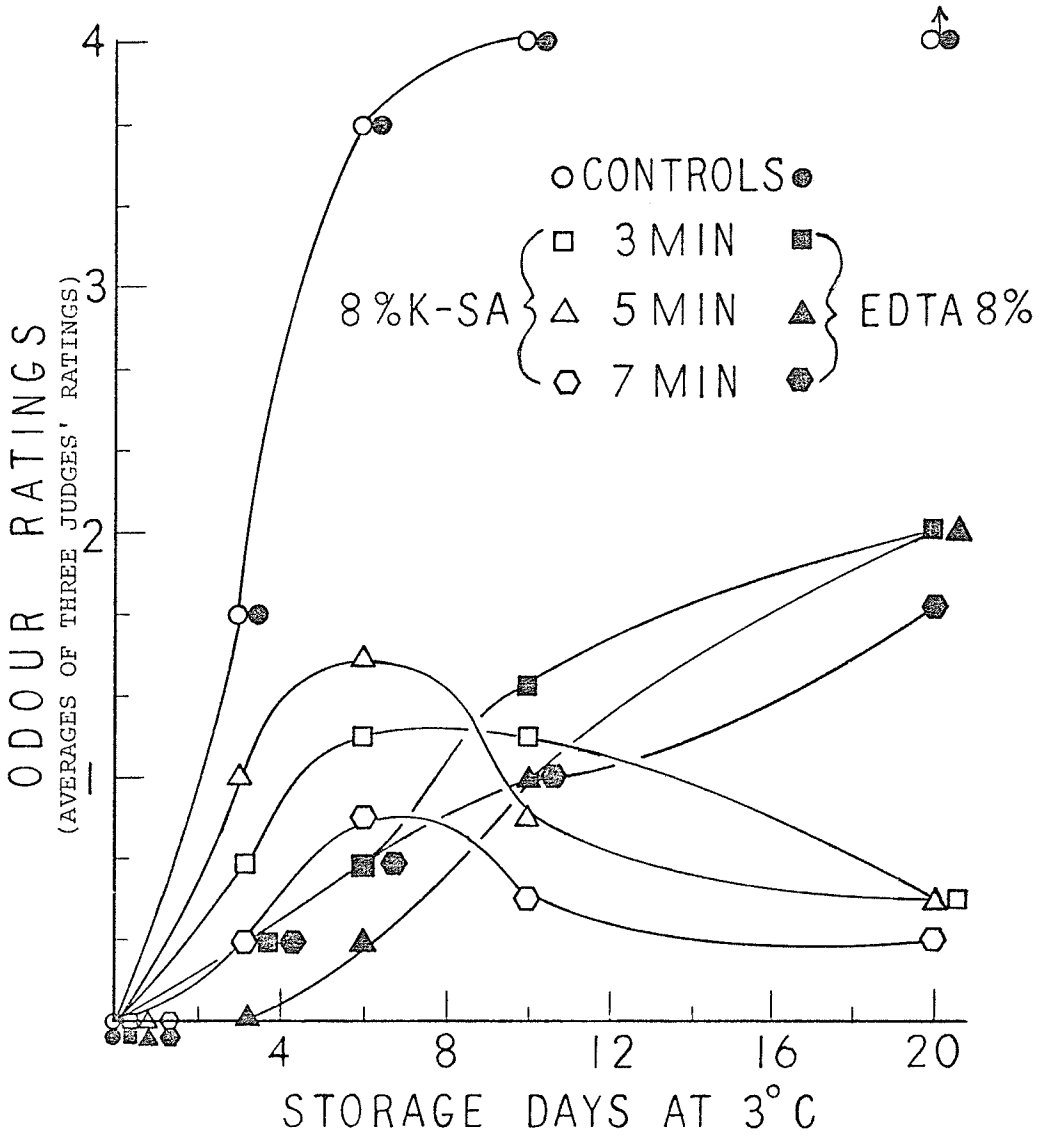


FIGURE 22 THE EFFECT OF POTASSIUM SORBATE AND DISODIUM EDTA AT VARYING EXPOSURE TIMES ON ODOUR RATINGS



with the 6 or 10% disodium EDTA levels and with the 8% potassium sorbate and combination treatments. However, the 8% EDTA treatments were observed to be significantly better than the 10% potassium sorbate treatments and significantly worse than the 6% potassium sorbate treatments (Appendix Tables 51 - 56).

The 10% EDTA treatments were observed to be significantly better than the controls; however there were no significant differences observed between the 10% EDTA treatments and the 6 and 8% EDTA levels, the 10% combination treatment, or the 6 and 8% potassium sorbate treatments. There was, however, a significant difference between the 10% EDTA and 10% potassium sorbate treatments at the 80 to 85% confidence interval. The values were close enough to the 90% level to make them somewhat significant (Appendix Tables 51 - 56).

Potassium sorbate treatments

In comparing the 6% potassium sorbate treatments to the other treatments it was observed that this level was significantly better, to a limited extent, than the 8 and 10% potassium sorbate and 10% combination treatments. There was no significant difference observed for the comparison with the 6 and 10% EDTA and combination treatments. It was observed, however, that the 6% potassium sorbate treatment was significantly better than the 8% EDTA treatment and

significantly better than the controls (Appendix Tables 51 - 56).

At the 8% level the 7 minute treatment was observed to be significantly better than the 5 and 3 minute treatments, but the 5 minute treatment was not significantly better than the 3 minute treatment (Figure 22 and Appendix Table 48). In comparing the 8% potassium sorbate treatment with the 6, 8 and 10% disodium EDTA treatments, and the 6 and 8% combination treatments no significant differences were observed. However, when the 8% treatments were compared with the 10% potassium sorbate treatments they were observed to be significantly better. The 8% potassium sorbate treatments were significantly worse than the 10% combination treatments and significantly worse than the 6% potassium sorbate treatments to a limited extent. All the 8% potassium sorbate treatments were significantly better than the controls (Appendix Tables 51 -56).

The 10% potassium sorbate treatments were observed to be significantly worse than the 6 and 8% EDTA and potassium sorbate treatments. The 10% treatments were worse than the 8 and 10% combination and 10% EDTA treatments only to a limited extent. There was no significant difference observed between the 10% potassium sorbate and 6% combination treatments, however the 10% potassium sorbate treat-

ments were significantly better than the controls (Appendix Tables 51 - 56).

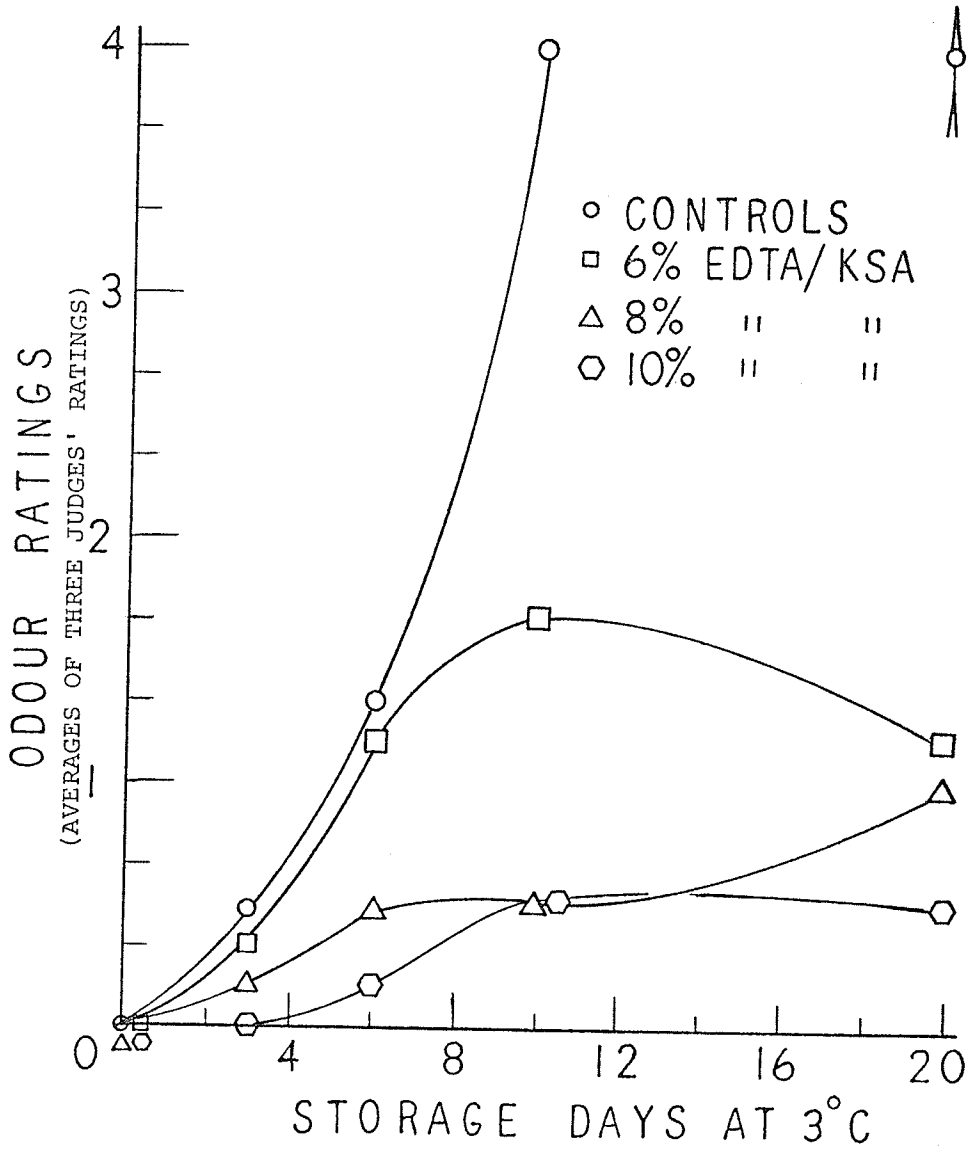
Combination treatments

All combination treatments showed significantly better results than the controls. The 6% combination treatments were not significantly better than the 6, 8 and 10% potassium sorbate and EDTA treatments. They were significantly worse than the 8 and 10% combination treatments (Appendix Tables 51 - 56).

The 8% combination treatments were significantly better than the 6% combination treatments but were not significantly better than the 10% combination treatments (Figure 23). There was no significant difference between the 8% combination treatments and the 8 and 10% EDTA treatments or the 8% potassium sorbate treatments. The 8% combination treatments were observed to be worse than the 6% EDTA and potassium sorbate treatments and 10% potassium sorbate treatments to a limited extent (Appendix Tables 51 - 56).

The 10% combination treatments were observed to be significantly better than the 6% combination, 8% EDTA, and 10% potassium sorbate treatments, and better than the 8% potassium sorbate treatments only to a limited extent. No significant differences were observed between the 10% combination treatments and the 6 and 10% EDTA, 6% potassium sorbate, and 8% combination treatments. Most significantly,

FIGURE 23 THE EFFECT OF A COMBINATION OF DISODIUM EDTA AND POTASSIUM SORBATE ON ODOUR RATINGS



the 10% combination treatment was not worse than any of the other treatments (Appendix Tables 51 - 56).

Organoleptic investigations

The taste panels showed that overall, the combination treatments produced the best results with the potassium sorbate treatments being second best and the disodium EDTA treatments being the third best, while the controls were consistently the worst. Table 5 shows the ranks and sums of ranks for the taste scores. On the basis of a trend in change of score from good to worse the 6 to 10% combination treatments showed the best results with 6% potassium sorbate and 8% EDTA treatments showing not much worse results than the 6 and 10% combination range. The 6 and 8% potassium sorbate samples were still acceptable after 14 days storage while the 4 and 10% samples were acceptable after from 10 to 14 days storage. The EDTA treatments were acceptable only after from 10 to 14 days storage. The 4% combination treatments were acceptable after from 10 to 14 days storage while the 6 to 10% combination treatments were still acceptable after 20 days storage. Controls became unacceptable after 7 days storage. Approximate shelf life extensions for these treatments from a taste standpoint are shown in Table 6.

Aroma scores showed that the best results were obtained with the combination treatments while the potassium

TABLE 5

TASTE SCORES, RANKS, AND SUMS OF RANKS FROM ORGANOLEPTIC STUDIES

DAYS OF STORAGE AT 3° C	TREATMENTS												
	% K-SA				% EDTA				% COMBINATION				
	4	6	8	10	4	6	8	10	4	6	8	10	CONTROLS
0	2	3	2	4	2	2	3	3	2	3	2	4	3
3	2	4	3	3	3	2	4	3	3	4	3	4	3
7	2	4	3	4	2	3	2	3	4	3	2	4	4
10	4	3	4	4	3	4	5	4	3	3	2	4	7
14	6	4	5	6	7	7	5	6	5	3	4	5	8
20	7	6	6	7	7	7	5	6	7	4	4	4	8+ *
RANKS	11	3	8	7	10	12	5	9	6	1	4	2	13
SUMS OF RANKS	29				36				13				

* 8+ This score went over the taste rating scale maximum.

TABLE 6

APPROXIMATE SHELF LIFE EXTENSIONS FROM A TASTE STANDPOINT

TREATMENTS	DAYS OF SHELF LIFE EXTENSION AT 3°C
Potassium sorbate:	
4 and 10%	5 Days
6 and 8%	10 Days
Disodium EDTA:	
4 and 6%	5 Days
8 and 10%	5 Days
Combination:	
4%	6 Days
6 to 10%	13+ Days*

* 13+ The actual shelf life extension would actually be more than 13 days.

sorbate treatments were second best. The EDTA treatments showed almost as good results as the potassium sorbate treatments. Controls consistently showed much worse results than all treatments. Table 7 shows the ranks and sums of ranks for the aroma trends comparisons. On the basis of a trend in change of score from good to worse the 4 to 10% combination treatments along with the 8% potassium sorbate and EDTA treatments showed the best results.

The 6 and 8% potassium sorbate treatments were acceptable after 14 to 20 days storage while the 4 and 10% potassium sorbate treatments were acceptable after 10 to 14 days storage. The 8 and 10% EDTA treatments were acceptable after 14 to 20 days while the 4 and 6% EDTA treatments were acceptable after 10 to 14 days storage. The 6 to 10% combination treatments were acceptable at 20 days while the 4% combination treatments were acceptable after 10 to 14 days storage. The controls were unacceptable after 7 days. Approximate shelf life extensions from an aroma standpoint are shown in Table 8.

Texture scores were all fairly acceptable up to 14 days storage. Controls were unacceptable before 7 days. All treatments were acceptable at 14 to 20 days except for 4 and 6% potassium sorbate and 4 and 8% EDTA treatments. Table 9 shows the texture scores obtained from taste panel testing.

General appearance scores mostly showed that all

TABLE 7

AROMA SCORES, RANKS, AND RANK SUMS FROM ORGANOLEPTIC STUDIES

DAYS OF STORAGE AT 3° C	TREATMENTS												
	% K-SA				% EDTA				% COMBINATION				
	4	6	8	10	4	6	8	10	4	6	8	10	CONTROLS
0	3	3	3	3	4	4	4	4	3	4	3	4	3
3	3	3	3	2	3	2	3	3	3	3	3	2	2
7	3	3	4	4	2	3	4	3	2	3	3	3	4
10	3	3	3	4	4	4	3	4	3	3	3	3	7
14	6	4	3	5	6	6	4	4	5	4	3	4	8+ *
20	8	7	5	5	8	7	6	7	5	4	3	4	8++ *
RANKS	11	7	3	9	12	10	5	8	6	2	1	4	13
RANK SUMS			30				35				13		

* 8+ 8++ These scores went over the rating scale maximum.

TABLE 8

APPROXIMATE SHELF LIFE EXTENSIONS FROM AN AROMA STANDPOINT

TREATMENTS	DAYS OF SHELF LIFE EXTENSION AT 3° C
Potassium sorbate:	
4 and 10%	5 Days
6 and 8%	10 Days
Disodium EDTA:	
4 and 6%	5 Days
8 and 10%	9 Days
Combination:	
4%	6 Days
6 to 10%	13+ Days *

* 13+ The actual shelf lfie extension would really be more than 13 days.

TABLE 9

TEXTURE SCORES FROM ORGANOLEPTIC STUDIES

DAYS OF STORAGE AT 3° C	TREATMENTS												
	% K-SA				% EDTA				% COMBINATION				
	4	6	8	10	4	6	8	10	4	6	8	10	CONTROLS
0	3	3	3	4	3	2	5	3	2	3	3	3	3
3	3	3	4	3	2	4	2	4	3	4	2	4	4
7	4	3	4	4	2	4	3	2	3	3	2	3	5
10	3	3	4	4	4	3	4	4	3	2	3	2	7
14	5	5	3	4	5	4	5	4	3	3	3	2	7
20	6	5	4	3	3	3	3	4	4	5	4	5	8

treatments showed acceptable results at between 14 to 20 days storage except for the 4% potassium sorbate treatments. Controls were unacceptable at 7 to 10 days. At 20 days the 4 and 6% potassium sorbate and the 6 and 10% EDTA treatments were unacceptable. Table 10 shows the general appearance scores obtained from the taste panels.

CHEMICAL EXAMINATION OF FILLET PIECES

Colorimetric Assay For Sorbic Acid Residuals In Fillet Pieces

The residual sorbate remaining on Whitefish fillet pieces as analyzed and calculated according to the Nury and Bolin method, is summarized in Table 11. It shows the approximate expected p.p.m. sorbate as residual when fillet pieces of approximately the same size (2 cm x 5 cm x 10 cm) and weight (25 gm) are dipped in the respective concentrations of potassium sorbate (indicated in weight per volume ratio) for an exposure time of five minutes.

Chemical analysis showed sorbic acid residuals to be in the range of from 445 p.p.m. for the 5% w/v potassium sorbate treatment for five minutes exposure time to 1410 p.p.m. for the 10% potassium sorbate for five minutes exposure time. The concentration which would give a residual concentration of 1000 p.p.m. would be in the range of from 8 to 9% w/v when exposure time is five minutes.

TABLE 10

GENERAL APPEARANCE SCORES FROM ORGANOLEPTIC STUDIES

DAYS OF STORAGE AT 3° C	TREATMENTS												
	% K-SA				% EDTA				% COMBINATION				
	4	6	8	10	4	6	8	10	4	6	8	10	CONTROLS
0	3	4	4	3	3	2	3	2	2	3	3	2	3
3	2	2	2	3	3	3	2	4	3	2	2	3	4
7	2	3	3	4	2	3	5	2	3	3	2	3	4
10	2	2	5	2	2	2	2	3	2	2	3	2	6
14	5	3	2	2	4	3	3	3	2	5	3	2	5
20	6	5	3	3	3	7	4	6	3	3	4	4	8

TABLE 11

THE EFFECT OF POTASSIUM SORBATE CONCENTRATION ON SORBATE RESIDUALS IN FILLET PIECES

CONCENTRATION	OPTICAL DENSITY		ug (STANDARD CURVE)		ug (CALCULATED)		P.P.M.		P.P.M. (AV'G)
5%	.328	.400	3.44	3.68	3.44	3.68	430	460	445
6%	.515	.492	5.60	5.36	5.60	5.36	700	670	685
8%	.357	.375	3.90	4.08	7.60	8.16	950	1020	985
9%	.450	.430	4.40	4.70	8.80	9.40	1100	1170	1135
10%	.525	.505	5.70	5.52	11.40	11.04	1440	1380	1410

5% and 6% determinations used a 250 ml volumetric flask.

8, 9, and 10% determinations used a 500 ml volumetric flask.

Colorimetric Determination Of EDTA Salts In Whitefish Fillet Pieces

The residual EDTA remaining on the fillet pieces as analyzed and calculated according to the Sinclair and Power method (1968), is summarized in Table 12. It shows the approximate expected values in p.p.m. EDTA residual when fillet pieces of the same above specifications were dipped in the respective concentrations of disodium EDTA (weight per volume ratio) for an exposure time of five minutes.

Observed EDTA residuals were in the range of from 480 p.p.m. for the 4% w/v for five minutes treatment to 1105 p.p.m. for the 10% w/v for five minutes treatment.

TABLE 12

THE EFFECT OF DISODIUM EDTA CONCENTRATION ON EDTA RESIDUALS IN FILLET PIECES

CONCENTRATION	OPTICAL DENSITY		mg EDTA/ 100 gm		P.P.M. EDTA		AV'G P.P.M.
4%	.325	.350	46	50	460	500	480
6%	.480	.505	72	68	720	680	700
8%	.615	.64	87	91	870	910	890
10%	.805	.755	107	114	1070	1140	1105

DISCUSSION

In the fish processing industry one of the most significant problem areas is the rapid spoilage of fresh fish fillets. In places where cooling and icing facilities have not been available, fish could only be kept a day or so after the catch and usually had to be sold the same day. In warm regions fish would be caught early in the morning and brought to the dock markets or fisheries. It would keep until the middle of the afternoon and by then they would have spoiled. Today icing and cooling make it possible to store fresh fish and fish fillets for up to five to seven days without seriously affecting organoleptic qualities.

It would then seem that the problem of keeping fish fresh until consumption, has been solved. Unfortunately, this is not the case. Fish processing plants usually are far removed from collection centers and often the market is far removed from the processing plants. This situation causes large gaps in the time it takes for the caught fish to reach the consumer. During this time spoilage occurs and the fish is no longer acceptable from an organoleptic point of view and also bacteriologically.

It has been proven that the initial spoilage is mainly due to bacterial spoilage processes. These bacterial pro-

cesses work initially to break down the fatty tissue as an immediate source of energy for further growth (Nair and Lahiry, 1968; Shewan, 1962). This fatty tissue breakdown causes the commonly encountered rancid odours. As spoilage progresses bacteria are responsible for the production of putrid and ammoniacal odours as the usuable supply of fatty material is exhausted.

The use of cold storage temperatures of from 1 to 5^{o}C delays bacterial spoilage for about five to seven days from catch time. After that time the psychrotrophic bacteria commonly found on the fish surface have increased in number and their metabolic products have accumulated significantly. It is these metabolic products which are responsible for the foul odours that result after five to seven days of cold storage. Pseudomonas putrefaciens, Pseudomonas fragi, and Pseudomonas spp. Group III and Group IV (Shewan, 1960) have been implicated as the main spoilage organisms (Levin, 1968; Castell et al., 1949; Silverio and Levin, 1967; and Castell and Greenough, 1958).

Once the main spoilage group of bacteria had been identified the search began for a chemical or combination of chemicals which could be used in conjunction with cold storage to prolong the useful storage life of fish fillets.

There are many preservative techniques applied to many food products and many of these techniques have been

applied to various forms of fish with varying degrees of success. However, most of these techniques may cause deleterious effects on the organoleptic qualities of the fish product in at least one of the organoleptic characteristics. Some of these processes are applicable to only certain forms of fish. For example, sorbitol and amino acid mixtures have been used effectively in fish sausages in low concentrations (Japanese Patent, 1970). Physical processes such as storage in controlled atmospheres of carbon dioxide described in work done by Shewan (1949) were found to be effective in extending the shelf life of fresh fish. However, the carbon dioxide had deleterious effects on the appearance of the fish.

Research literature revealed the possibility of using potassium sorbate and EDTA either separately or together in combination to preserve and extend the shelf life of fresh fish fillets.

Whitefish fillets were chosen to work with, because they are particularly subject to bacteriologically induced rancidity due to their high fat content and because they were readily available from the Fresh Water Fish Marketing Corporation.

In the course of the bacteriological studies it was found that initial counts were at a level of from 1.0×10^3 to 1.0×10^4 organisms per gram. As storage time increased it was found that a level of approximately 1.0 x

10^8 organisms per gram in the controls corresponded very closely with the formation and definite detection of rancid odours. Thus, a level of 1.0×1.0^8 viable psychrotrophic organisms per gram ('10^8 level') was chosen as the acceptable limit. Any significant counts over this amount would indicate a spoiled sample. This method of testing freshness was chosen over more sophisticated testing procedures such as the Spinelli (1964) hypoxanthine estimation technique because of its simplicity. Lerke and Farber (1969) found that the former means of testing correlated well with the latter technique.

It should be noted that at a level of 10^8 organisms per gram in the treated samples, rancid odours of the same degree as in the controls of the same storage time were either not observed at all or were almost always observed to a lesser degree where detected at all.

Using the 10^8 level as the acceptability limit for both controls and treatments, the shelf life extension statistic was derived arbitrarily by using the difference in the number of days the treatments required to reach the 10^8 level as compared with the number of days the controls required to reach the 10^8 level. With this information we could compare shelf life extensions from a bacteriological viewpoint with shelf life extensions from an organoleptic viewpoint.

Chemical treatment of the fillets was chosen as

the simplest and most economical method of preservation. The object of the experimental investigations was to decrease initial bacterial load and to inhibit subsequent bacterial growth and odour forming metabolic reactions. This had to be accomplished in such a way as to not significantly harm organoleptic characteristics. Dips in solutions of EDTA and potassium sorbate, in varying concentrations and for varying exposure times were chosen as the experimental method of treatment. Since EDTA has good antioxidant qualities and is commonly used in the food industry for this purpose (Boyd and Southcott, 1968; Pelroy and Seaman, 1969; Power et al. 1968; Regier and Longard, 1970; Levin, 1967), it was employed with the hope that bacterial production of oxidative rancidity would be significantly inhibited. Potassium sorbate is another chemical preservative widely used in the food industry today (Borgstrom, 1969). Devebere and co-workers (1972) found sorbic acid to have significant inhibitory effects on the growth of psychrotrophic fish spoilage bacteria such as Pseudomonas spp.

The chemical treatments used in this work were potassium sorbate in the concentration range of from 0.25 to 10.0% w/v and EDTA from 1 to 10% w/v. The exposure times were from 10 seconds to 7 minutes. Generally, in the lower concentration ranges of up to 4 to 5% somewhat significant results were observed, however, shelf life extensions were small. This could have been due to insufficient exposure

time as it would be expected that a slightly lower concentration at a longer exposure time might work equally as well as a higher concentration at a shorter exposure time. However, this is not necessarily true. As indeed this work indicated, there was no significant difference among exposure times over the range of from three to seven minutes. There was, however, some small differences between short times such as 10 seconds and long times such as seven minutes, but these differences were not consistent. This would indicate that the effectiveness of the treatment does not critically depend on exposure time but rather on concentration. It confers a surface rather than subsurface protection. Therefore, a spray might prove to have the same effect as a dip and would have the added advantage of taking less time to carry out the treatment.

At the 5% potassium sorbate level shelf life extensions of approximately 4.5 to 5.5 days were observed while residuals left on the fish were approximately 450 p.p.m. There was a noticeable difference in viable counts for the treatments from 4 to 6% w/v potassium sorbate as compared with controls. Shelf life extensions of from 3.5 to 15 days were observed in this range. These results together with their graphs (Figures 8, 9, 10, 11) indicate that there is a significant difference among treatments in the concentration range of from 4 to 6% w/v. Furthermore, the 6% treatment results show the best results of all with

a shelf life extension of approximately fifteen days and residuals of approximately 730 p.p.m. sorbate. In all cases of 4 to 6% there was an initial decrease in counts followed by increasingly lower rates of growth as concentrations increased.

At approximately 8% w/v potassium sorbate for five minutes exposure time the residual level began to reach and exceed 1,000 p.p.m. which is the legal safe maximum level. Although the results and shelf life extensions for 8% and for 10% w/v potassium sorbate were better than for 6% they could not be considered as useful as the 6% level of treatment because they left residuals over the safe maximum limit. Bacteriological shelf life extensions for the 8 and 10% treatments for a five minute exposure time were estimated to be over twenty and twenty-five days, respectively. At levels of treatment over 8% w/v potassium sorbate it seems that the chemical has a killing effect on a large proportion of the bacterial population and its inhibitory effect on the survivors is significant enough to keep levels constant over a period of twenty days.

One possible explanation of its activity is that the sorbic acid, as a weak acid, in high concentration changes the surface pH making it undesirable for bacterial growth. Sorbate discourages the enzymatic production of more free fatty acids from fat tissue by the bacteria.

Without this means of energy production the bacteria present cannot grow unless they find an alternate energy supply or are able to somehow circumvent this inhibition. A similar explanation was offered by Emard and Vaughn (1952). The operating mechanism of sorbic acid works in such a way as to block the normal functioning of some sulfhydryl group containing enzymes. Therefore it would inhibit a variety of enzymic breakdown reactions, not only fat oxidation reactions but also protein degradation reactions. Sorbic acid is a competitive inhibitor similar in structure to fatty acids which are naturally responsible for 'turning off' fat oxidizing enzymes when they are no longer needed. This latter fact is particularly apparent when the metabolic products such as free fatty acids begin to build up and the supply of nutrients begins to dwindle.

The disodium calcium salt of EDTA did not yield as good results as did the disodium EDTA salt. Since EDTA activity is that of a chelator, the calcium in the former salt of EDTA tends to discourage chelating, which results in a lowered antioxidant potency. The disodium salt is still considered safe to use in some foods in spite of many authorities insisting that it can bind dangerously large amounts of calcium in fish muscle, for example, and make it unavailable to the human diet. Levin (1967) found that a 300 p.p.m. residual of disodium EDTA would chelate 13 to 40%

of the available calcium present in the fish muscle.

The odour analysis was very important in that subjectively this technique is the best way to determine whether fish is fresh or is not fresh. The average consumer has only their senses to guide them in their freshness judgments. The rating scale in Table 3, page 50 was used to statistically compare treatment effects.

The odour analysis results indicate that all treatments have significantly better results than controls but it would be difficult to accurately describe any one of the treatments as being consistently better than another.

At the same concentration and exposure time all three different types of treatments were observed to have similar effects except at the 10% level where potassium sorbate treatment was significantly worse than the 10% EDTA and combination results. Varying the concentrations of each of the three types of chemical treatments did not seem to give significantly different results. However, when comparisons were made between various types and concentrations differences became more apparent. In these comparisons the EDTA treatments seemed to give better results than potassium sorbate treatments as the concentrations approached the 10% w/v level. This may have been the consequence of different sensory detection levels for the two chemicals. The panelists

observed an odd smell in treatments of high sorbate concentrations. When cross-comparing different types of chemical treatments between high and low concentrations ranges obvious differences were observed. For example, a distinct difference was seen between the 10% combination treatments and both the 8% EDTA and potassium sorbate treatments. The former treatment was observed to be significantly better than the latter treatment. However, there seemed to be no significant difference between the 10% combination and 6% EDTA and potassium sorbate treatments. A possible explanation of this phenomenon is that although the normally expected result is increased efficacy with an increase in concentration, there seems to be a particular balance between beneficial chemical effects and harmful effects. On the one hand an increased concentration could benefit the inhibition of oxidation thus resulting in improved odour results over storage time. However, on the other hand this increased chemical concentration increases the likelihood of detection of off-odours due to the addition of the chemical itself. The 8 and 10% treatments are close enough together in concentration so that differences in effects are noticed on one scale only, that of the inhibition of odour-forming activities so that a slightly higher concentration has a slightly better effect. In comparing the 10% potassium sorbate treatments with the 6% treatments, the probable result would be

similar effects in that the 10% concentration although having better odour inhibiting effects than the 6% level, contributes to odour in itself, thus causing an equalizing effect between the two different treatments.

In conclusion it could be said that at the 6% level all three treatments gave the best results from a residual standpoint. They all had similar inhibitory effects on the retardation of odour formation.

The taste panel results on the cooked fish showed more differences among treatments over the length of the storage period. The combination results along with the 8% potassium sorbate and the 6% EDTA results showed the best taste scores while the combination results and the 8% EDTA and potassium sorbate treatments showed the best aroma scores.

The 10% potassium sorbate scores were not as good as the 8% scores due to the detection of undesirable chemical tastes and aromas. However, the 8% scores were better than the 4 and 6% results because the increased concentration had increased inhibitory effects on the formation of off-flavours and off-odours due to chemical and microbial deterioration. This concentration was not as high as the 10% treatments therefore chemical tastes and odours were not apparent.

In combination, the EDTA and potassium sorbate

seemed to have synergistic action in that together they seemed to work better than either of the two alone. The rank sums of the taste and aroma scores as shown in Tables 5 and 7 indicate that the results of the combination treatments were twice as good as either of the other two treatments. This would then tend to rule out the possibility of simply an additive effect of the two other treatments being the sole reason for the better combination results. The expected rank sums for the combination treatments would not be expected to be much better if an additive effect were solely the case. Rather, in addition to the additive effects there seems to be a synergism between the EDTA and potassium sorbate treatments which results in the combination treatments being so much better than either of the two alone.

It is sometimes difficult to describe an appropriate theoretical model for an experiment. The real difficulty often comes after the model has been defined. That difficulty is in finding the probabilities associated with the model. For this reason the model is changed slightly in order to be able to solve for the desired probabilities. This must be done in such a way as to not change the model too drastically in order that the model still be realistic. Thus it should be possible to obtain exact solutions for these approximate problems. This area of statistics is cal-

led 'parametric statistics' and employs such tests as the 't' test and the 'F' or 'Fisher' test. These two tests were used to analyze the microbiological data of this thesis work.

The 't' test analysis was applied to tests where paired samples were used in the microbiological analysis. This method is very accurate and supplies exact information where required. The range of concentration and exposure times explored with this method of analysis provided information on how further analysis in higher concentration ranges should be made. In brief, the studies undertaken using this method of analysis were actually rangefinder studies.

The rationale for pairing was that two strips of fish lying alongside each other had to be very similar in all respects such as biochemical activity, microbial population, activity, density, and type. Therefore fillet to fillet variation and piece to piece variation within the same fillet could not interfere much with what had to be the most true results.

The series of microbiological experiments which used the analysis of variance statistical method or the Fisher test were in the higher concentration range and one experimental block included all chemical types of treatments. The previous method of analysis usually only included one chemical treatment in any experimental pair or block. The

second method of analysis, although not more accurate than the first, provided much more information with the use of much lower sample population numbers.

Non-parametric statistics embodies the search for approximate solutions to exact problems. These methods require less computational work than most parametric test analyses and therefore are easier and quicker to apply than other statistical methods. The following two tests were used for the analysis of odour testing data and taste panel results for aroma and taste, respectively.

The Wilcoxon Signed Ranks Test was chosen for the analysis of the odour testing data due to its simple approach to a very complex subjective testing procedure. It provided the most meaningful and significant results when applied to this data in comparison with other more complex testing procedures.

The Cox-Stuart test for trend was chosen to be used for the aroma and taste data. It was chosen because it could be adapted to a study of how one treatment could tend to be more or less effective at preservation in a useful way than others over a certain period of storage time. This procedure took into account not only day to day trends but trends over the entire length of the study.

New chemicals and combinations of chemicals and processes are being developed every year for the preservation

of fish. It must however be kept in mind that preservation is not always the most desirable substitute for efficient transportation systems and well designed carriers which transport the product to the market without chance for spoilage. In the consumer's mind, freshness is still prominent and that means a product as close as possible to the original state of the fish in all respects, without the addition of preservatives. Food processors must bear the responsibility for future generations for the way in which processed foods are manufactured. Preservatives must be thoroughly and exhaustively tested for all possible human side effects before being allowed to be used in a processed food item.

The outcome of these studies indicates that there is a possibility of using potassium sorbate, disodium EDTA, or more likely a combination of the two chemicals as a fish fillet preservative. However further studies are required on a larger pilot scale in order to fully determine how effective such a treatment would be on a commercial scale. Considering that the two chemicals are already widely used as food preservatives, their availability and reasonable cost would probably prove to be attractive to the fish processing industry from both an economic and practical usage standpoint.

SUMMARY

Potassium sorbate has an inhibitory effect of the growth of spoilage bacteria on fresh whitefish fillets. In higher concentrations of from 8 and 10% w/v it has an initial killing effect.

Potassium sorbate demonstrates antioxidant activity. This activity is not however, as good as EDTA's antioxidant activity at concentrations from 6 to 10% w/v.

Shelf life extensions of from one to six days are possible with the treatment range of from 0.05 to 5.0% w/v potassium sorbate. Extensions of from ten to twenty-five days were observed with the treatment range of from 6 to 10% w/v potassium sorbate.

Potassium sorbate is acceptable from an organoleptic point of view in treatment concentrations as high as 8%. However, at 10% in samples stored for over ten days typical sour-acid tastes become apparent. Shelf life extension from an organoleptic point of view was determined to be about 5 to 6 days for the 4% level to approximately 10 to 11 days for the 8% level of treatment.

EDTA has an inhibitory effect on the growth of spoilage bacteria on fresh whitefish fillets. In higher concentrations of from 8 to 10% it demonstrates an initial killing effect. However, these effects are not as pronounced as those observed with the potassium sorbate treatments at the same concentrations.

The antioxidant activity of EDTA is more pronounced than that of potassium sorbate at the same concentration.

Shelf life extensions of from two days for a 4% w/v disodium EDTA treatment to approximately 19 days for the 10% level of treatment were observed.

EDTA proved to be acceptable from an organoleptic point of view in treatment concentrations as high as 10% w/v. However, samples tested at 20 days storage, over the range of concentrations of from 4 to 10%, did not seem to be nearly as acceptable as the combination treatments and in most cases were a little less acceptable than the potassium sorbate treatments except for the 10% level. From an organoleptic point of view shelf life extensions were observed to be approximately 4 to 5 days for the 4% level up to 5 to 6 days for the 10% level.

The combination treatments had the most significant effects on the growth of spoilage bacteria. Kill effects were observed at lower concentrations than those required for kill using either of the two chemicals separately.

Antioxidant activity was very well pronounced and was better than either of the two separate chemical treatments.

Shelf life extensions were over twenty-five days for the 6% treatments.

The combination results were the most acceptable from an organoleptic point of view. Shelf life extensions of from six days for a 4% combination treatment up to over 13 days for a 6% combination treatment were observed.

BIBLIOGRAPHY

American Public Health Association (1967) Standard Methods For The Examination Of Dairy Products, 12th ed, USA pp. 71-87

Ando, N. and Nagata, Y. (1971) Effects of some food preservatives on the behaviour of nitrite during the processing of meat products. Proceedings Of The European Meeting Of Meat Research Workers vol 15 pp. 474-81

Annual Statistical Review Of Canadian Fisheries, 1957-72(Ottawa, 1973) vol 5 p. 33

Banks, A. (1937) Rancidity in fats. Journal Of The Society Of Chemical Industries vol 56 pp. 13-15T

_____, Keay, J.N., and Smith, J.G.M. (1957) Structure of conjugated methyl linoleate hydroperoxide. Nature vol 179 p. 1078

Borgstrom, G. (1969) Principles Of Food Science vol 1 Food Technology, Chapters 7 to 12 (Toronto, The MacMillan Co.) pp. 148-325

Boyd, J.W. and Southcott, B.A. (1968) Comparative effectiveness of ethylenediaminetetraacetic acid and chlortetracycline for fish preservation. Journal Of The Fisheries Research Board Of Canada vol 25 pp. 1753-58

Braekkan, O.R. and Boge, G. (1964) Proceedings Of The International Symposium On Food Microbiology vol 4 pp. 271-78

Brock, T.D. (1958) The effect of salmine on bacteria. Canad-

ian Journal Of Microbiology April pp. 65-71

Castell, C.H., Richards, J.F., and Wilmot, I.(1949) Pseudomonas putrefaciens from cod fillets. Journal Of The Fisheries Research Board Of Canada vol 7 pp. 430-31

_____, and Greenough, M.F. (1959) The action of Pseudomonas on fish muscle. Part IV: Relation between substrate composition and the development of odours by Pseudomonas fragi. Journal Of The Fisheries Research Board Of Canada vol 16 pp. 21-31

_____, MacLean, J., and Moore, B. (1965) Rancidity in lean fish muscle. Part IV: Effect of sodium chloride and other salts. Journal Of The Fisheries Research Board Of Canada vol 22 p. 4

Chai, T., Chen, C., Rosen, A., and Levin, R.E. (1968) Detection and incidence of specific species of spoilage bacteria on fish. Applied Microbiology November pp. 1738-41

Chan, M.S. and Yang, S.S. Summaries of Toxicology of EDTA. Food And Cosmetics Toxicology vol 2 pp. 763-67

Chen, T.P. and Fellers, C.R. (1926) Fish preservation by hypochlorites. Univerity Of Washington Fisheries Publications vol 1 pp. 205-27

Commercial Fisherman's Guide (Department Of Natural Resources Saskatchewan, Canada, 1971) pp. 26-28

Conover, W.J. (1971) Practical Non-Parametric Statistics (pub. J.R. Wiley and Sons, Toronto) pp. 121-39

Corlett, J.R., Lee, J.S., and Sinnhuber, R.O. (1965) Application of replica plating and computer analysis for rapid identification of bacteria in some foods. Part III: Analysis of microbial flora in irradiated Dover sole. Applied Microbiology September pp. 818-22

Devebere, J.M. and Voets, J.P. (1970) The effect of potassium sorbate and citric acid on the production of TMA and ammonia in prepackaged dogfish. Voedingsmiddelentechnologie (Belgium) vol 1(50) pp. 561-64

_____, Voets, J.P., and Pudjo Tjiptono, A. (1972) The effect of sorbic acid on the growth of triaminooxidase and urease containing bacteria isolated from fresh fish. Mededelingea van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent. vol 36(2) pp. 555-60

Delisle, A.L. (1969) Studies on the bacteriophages of psychrophilic spoilage bacteria. Dissertation Abstracts International Section B: The Sciences and Engineering (University of Amherst, Massachusetts, USA) vol 30(2) pp.754-55

Diehl, J.F. (1969) Irradiation of Food: A Review. Revista del Frio.(Spain) vol 14(3) pp.139-46

Durrant, N.W. (1968) Major advances in fish processing technology. National Fisherman vol 49(13) pp. 107-108

Emard, L.O. and Vaughn, R.H. (1952) Selectivity of sorbic acid media for the catalase negative lactic acid bacteria and Clostridia. Journal Of Bacteriology vol 63 pp. 487-94

Evelyn, T.P.T. and McDermott, L.A. (1961) Bacteriological studies of fresh water fish. Canadian Journal Of Microbiology vol 7 pp. 375-81

Fletcher, T.C. (1968) Carbohydrate polypeptide polymers of epithelial secretions and connective tissue of fish. PhD Thesis, University of Aberdeen, UK, Fisheries Biochemical Unit

Frazier, W.C. (1967) Preservatives used on fish. Food Microbiology (McGraw-Hill, USA) Chapter 17 p. 288

French Patent (1970) # 587-601/1970

Freshwater Fish Marketing Corporation (Manitoba) Annual Report, 1970-71 pp. 4, 9, 19

Gillespie, D.C. and Ostovar, K. (1971) Effects of washing fish on keeping quality. Journal Of The Fisheries Research Board Of Canada vol 28 pp. 783-85

Gibbs, W.E. (1923) Germicide in ice to allay decay. Canadian Fisherman vol 10 pp. 99-100

Gray, C.W. and Wilkinson, S.G. (1965) The action of ethylenediaminetetraacetic acid on Pseudomonas aeruginosa. Journal Of Applied Microbiology vol 28 p. 153

Haraguchi, T. and Iimori, M. (1970) Cleaning of fish with surface active agents. Bulletin Of The Japanese Society Of Scientific Fisheries vol 36(5) pp. 497-501

Ingram, M., Ottaway, F.J.H., and Coppock, J.B.M. (1956)

Preservative action of acid substances in food. Chemical Industry (London) vol pp. 1154-63

Jadhav, M.G. and Magar, N.G. (1970) Preservation by freezing and glazing. Part II: Keeping quality of fish with particular reference to yellow discoloration and other allied changes on prolonged storage. Fishery Technology vol 7 (2) pp. 146-49

Japanese Patent (1970) # 17/575/70 (Taiyo Fishery Co.,Ltd.)

_____ (1971) # 6080/71

Krishnaswamy, M.A. and Rudra Setty, F.M. (1966) Orange oil as a preservative for fish sausage. Bulletin Of The Japanese Society Of Scientific Fisheries vol 32(II) pp. 972-75

Kuusi, T. and Loytomaki, M. (1972) Effects of EDTA on fresh fish. Zeitschrift Fur Lebensmitteluntersuchung und Forschung vol 149(4) pp. 196-204 (Technical Research Center, Food Research and Technology Lab, Otaniemi, Finland)

Lee, J.S. and Price, R.J. (1969) Inhibition of food spoilage Pseudomonas by Lactobacillus species. Bacteriological Proceedings vol 1969 p. 13

Lerke, P., Adams, R., and Farber, L. (1965) Bacteriology of the spoilage of fish muscle. Part III: Characterization of the spoilers. Applied Microbiology July pp. 625-30

Levin, R.E. (1967) The effectiveness of EDTA as a fish preservative. Journal Of Milk And Food Technology vol 30 pp. 277-83

Levin, R.E. (1968) Detection and incidence of specific spoilage bacteria on fish. Part I: Methodology. Applied Microbiology vol 16 pp. 1734-37

Liston J. and Matches, J.R. (1969) Study of the basic microbiological and biochemical factors involved in the irradiation of marine products. Food Irradiation Contractors Meeting (Atomic Energy Commission, USA, University of Seattle) pp. 77-81

Lucke, F.R. and Schwartz, W. (1938) Microbiological studies on seafish. Microbiological Archives (West Germany) vol 8(2) pp. 207-30

Luck, E. (1970) The use of sorbic acid in food preservation. Australian Food Manufacture vol 39(8) pp. 28-32

Mendellhall, W. (1971) Introduction to Probability and Statistics 3rd ed (Wadsworth Publishing Co., USA) pp. 224-48 and pp. 332-36

Metzner, H., Hutschenreuter, R., and Oeser, H. (1938) Keeping seafish fresh. Vorratspflege v. Lebensmittelforschung vol 1 pp. 613-22

Michael, G.T., Davies, R., Silverman, G.J. (1968) Selective influence of carbon dioxide and irradiation on the microbial flora of haddock. Bacteriological Proceedings vol 1968 p. 10

Morita, M., Fujimaki, M. (1972) Effects of copper and EDTA on the autooxidation of phospholipid emulsions. Agricultural and Biological Chemistry vol 36(7) pp. 1163-67

Nair, R.B. and Lahiry, N.L. (1968) Factors affecting the quality of fresh fish and its retention by chilling. Journal Of Food Science and Technology(Mysore, India) October vol 5(3) pp. 107-116

Nury, F.S. and Bolin, H.R. (1962) Colorimetric assay for potassium sorbate in dried fruits. Journal Of Food Science vol 27 pp. 370-72

Pelroy, G.A. and Seaman(Jr.), J.P. (1969) Effect of EDTA on the spoilage characteristics of Petrale sole and ocean perch fillets. Journal Of The Fisheries Research Board Of Canada vol 26 pp. 2651-57

Power, H.E., Sinclair, R., and Savagon, K. (1968) Use of EDTA compounds for the preservation of haddock fillets. Journal Of The Fisheries Research Board Of Canada vol 25 pp. 2071-82

Price, R.J. and Lee, J.S. (1970) Inhibition of Pseudomonas spp. by hydrogen peroxide producing Lactobacilli from seafood and other marine sources. Journal Of Milk And Food Technology vol 33(1) pp. 13-18

Regier, L.W., Laycock, R.A., and Longard, A. (1970) EDTA for iced fillet preservation. Journal Of The Fisheries Research Board Of Canada Technical Report # 214

Salmon, J. and LeGall, J. (1936) Use of ozone to maintain the commercial fresh life of fish. Rev. Gen. Refroid. (France) vol 17 pp. 317-22

Sen, N.P., Smith, D.C., Schwinghamer, L., and Howsam, B. (1970) Formation of nitrosamines in nitrite treated fish. Journal Of The Canadian Institute Of Food Technology vol 3(2) pp. 66-69

Shaw, M.K. and Nicol, D.J. (1969) Effect of the gaseous environment on the growth on meat of some food poisoning and food spoilage organisms. Proceedings Of The European Meeting Of Meat Research Workers vol 15 pp. 226-32

Shewan, J.M. (1949) Some bacteriological aspects of handling , processing, and distribution of fish. Journal Of The Royal Sanitation Institute (UK) vol 69 pp. 395-421

_____, (1961) The microbiology of sea-water fish. Fish As Food vol 1 Ed G. Borgstrom pub. Academic Press, New York p. 489

_____, (1962) The bacteriology of fresh and spoiling fish and related chemical changes. Recent Advances In Food Science vol 1 p. 167

_____, (1970) Bacteriological standards for fish and fishery products. Chemistry and Industry (UK) vol 6 pp. 193 -199

_____, (1971) The microbiology of fish and fishery products : A progress report. Journal Of Applied Bacteriology vol 34(2) pp. 299-315

_____, Hobbs, G., and Hodgekiss, W. (1960) The Pseudomonas and Achromobacter groups of bacteria in the spoilage of

marine whitefish. Journal Of Applied Bacteriology vol 23 pp. 463-68

Shtenburg, A.J. and Ignat'ev, B.D. (1970) Toxicological evaluations of some combinations of some food preservatives . Food And Cosmetics Toxicology (USSR) vol 8(4) pp. 369-380

Silverio, R. and Levin, R.E. (1967) Evaluation of methods for determining the bacterial population of fresh fish fillets. Journal Of Milk And Food Technology vol 30 pp. 242-46

Spinelli, J., Eklund, M., and Miyauchi, D. (1964) Measurement of hypoxanthine in fish as a method of assessing freshness. Journal Of Food Science vol 29 pp. 710-14

Tanikawa, E. and Shitamori, I. (1963) Cold storage of cod fillets treated with polyphosphates. Food Technology vol 17(11) pp. 1725-30

Tarr, H.L.A. (1944) Antioxidants and prevention of rancidity in certain Pacific coast fish. Nature vol 154 pp. 824-26

____, (1954) Microbiological deterioration of fish postmortem, its detection and control. Bacteriological Reviews vol 18 pp. 1-15

____, (1961) Chemical control of microbiological deterioration. Fish As Food vol 1 Ed G. Borgstrom pub. Academic Press, New York p. 489

Tarr, H.L.A. and Sunderland, P.A. (1940) The comparative value of preservatives for fresh fish fillets. Journal Of The Fisheries Research Board Of Canada vol 5(2) pp. 148-63

United States Patent (1970) # 3/563/770 (T.E. Furia-Geigy Chemical Corporation)

United States Patent (1971) # 3/622/351-1971

Wallhauser, K.H. and Luck, E. (1972) Antibacterial activity of sorbic acid. Deutsche Lebensmittel-Rundschau vol 68 (2) pp. 39-44 (German Federal Republic)

West German Patent Application (1971) # 767/438-1971

Yamada, K. and Murata, A. (1949) On the effect of sodium nitrate in preserving fish meat. Bulletin Of The Japanese Society Of Scientific Fisheries vol 15 pp. 380-84

APPENDICES

APPENDIX TABLE 1

THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C					
	0 DAYS *		3 DAYS			6 DAYS **
	SPC/gm.	YEASTS/gm.	SPC/gm.	YEASTS/gm.	MOLDS/gm.	SPC/gm.
CONTROLS	1.25×10^5	3.3×10^5	----------			----------
	3.4×10^4	0	6.0×10^6	0	0	
	2.3×10^4	5.0×10^1	----------			----------
0.05% / 10 sec	1.51×10^5	1.05×10^3	4.8×10^6	0	0	All counts were at approx- roximately the level of 5.0×10^7 cells/gm.
0.05% / 30 sec	1.0×10^4	1.57×10^3	8.6×10^6	0	0	
0.05% / 1 min	1.7×10^4	0	6.0×10^6	0	0	
0.05% / 2 min	3.3×10^4	5.3×10^2	7.0×10^6	6.9×10^2	7.0×10^1	
0.10 / 10 sec	8.8×10^4	9.4×10^2	1.68×10^6	0	0	
0.10% / 30 sec	1.5×10^5	2.2×10^3	4.6×10^6	0	0	
0.10% / 1 min	1.0×10^5	0	6.2×10^6	0	0	
0.10% / 2 min	1.37×10^5	8.8×10^2	7.7×10^6	0	0	

* All mold counts were zero and therefore were not entered.

** All yeast and mold counts were zero and therefore were not entered.

APPENDIX TABLE 2

THE EFFECT OF VARYING CONCENTRATIONS OF POTASSIUM SORBATE AND VARYING EXPOSURE TIMES ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C			
	0 DAYS	1 DAY	3 DAYS	6 DAYS
	SPC/gm.	SPC/gm.	SPC/gm.	SPC/gm.
0.25% / 1 min	5.1×10^3	5.79×10^4	3.81×10^6	6.5×10^7
0.25% / 2 min	4.3×10^3	2.38×10^4	1.52×10^6	1.2×10^8
0.25% / 3 min	3.2×10^3	1.06×10^4	2.05×10^6	9.8×10^7
CONTROLS	5.2×10^3	7.02×10^4	3.45×10^6	2.15×10^8
0.50% / 1 min	1.52×10^5	1.45×10^5	1.18×10^6	1.88×10^8
0.50% / 2 min	1.26×10^5	2.24×10^5	1.64×10^6	1.23×10^8
0.50% / 3 min	6.3×10^4	1.27×10^5	4.75×10^5	5.8×10^7
CONTROLS	2.61×10^5	3.6×10^5	3.97×10^7	2.26×10^8

APPENDIX TABLE 3

THE EFFECT OF POTASSIUM SORBATE FOR VARYING EXPOSURE TIMES

ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C		
	0 DAYS	4 DAYS	8 DAYS
	SPC / gm	SPC / gm	SPC / gm
Controls	3.7×10^4	1.83×10^7	1.3×10^9
0.70% / 3 min	4.2×10^4	5.8×10^6	7.9×10^8
0.70% / 5 min	7.0×10^4	7.5×10^6	9.8×10^8
0.70% / 7 min	2.1×10^4	4.4×10^6	8.5×10^7

APPENDIX TABLE 4

THE EFFECT OF VARYING CONCENTRATIONS OF POTASSIUM SORBATE FOR A FIXED EXPOSURE TIME ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C		
	0 DAYS	8 DAYS	14 DAYS
	SPC/gm	SPC/gm	SPC/gm
Controls	2.55×10^5	1.84×10^9	1.0×10^{10}
0.5% / 5 min	2.52×10^5	9.75×10^8	9.0×10^9
1.0% / 5 min	1.87×10^5	7.5×10^8	7.0×10^9
3.0% / 5 min	2.47×10^5	1.95×10^8	5.0×10^9
5.0% / 5 min	2.02×10^5	2.95×10^7	2.42×10^9

Fisher test statistic is 0.136177

Student's t-test statistic is 1.67094

* Above results are not very significant except those for the 5% / 5 min treatments.

APPENDIX TABLE 5

THE EFFECT OF DISODIUM CALCIUM EDTA ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C		
	0 DAYS	5 DAYS	9 DAYS
	SPC/gm	SPC/gm	SPC/gm
Controls	3.89×10^5	8.83×10^6	3.05×10^9
0.5% / 5 min	1.48×10^5	6.63×10^6	2.14×10^9
0.75%/ 5 min	4.85×10^5	6.88×10^6	2.41×10^9
1.0% / 5 min	8.1×10^4	1.32×10^7	2.19×10^9
1.5% / 5 min	6.85×10^4	5.0×10^6	1.78×10^9

Fisher test statistic is 0.01848 (no significance)

APPENDIX TABLE 6

THE EFFECT OF DISODIUM CALCIUM EDTA ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C			
	0 DAYS	6 DAYS	10 DAYS	14 DAYS
Trial 1	SPC/gm	SPC/gm	SPC/gm	SPC/gm
Controls	9.2×10^{4}	5.45×10^{7}	1.65×10^{8}	3.0×10^{10}
4% / 5 min	1.13×10^{5}	2.76×10^{7}	5.75×10^{8}	2.74×10^{9}
Trial 2				
Controls	1.40×10^{5}	5.5×10^{7}	6.8×10^{8}	7.65×10^{9}
4% / 5 min	3.57×10^{4}	3.57×10^{4}	5.6×10^{8}	4.0×10^{9}

Student's t-test statistic is 1.3785 . Since the critical value for the 90% confidence level is 1.638 the results therefore are insignificant.

APPENDIX TABLE 7

THE EFFECT OF POTASSIUM SORBATE FOR VARYING EXPOSURE TIMES ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C		
	0 DAYS	5 DAYS	9 DAYS
	SPC/gm	SPC/gm	SPC/gm
Controls	3.14×10^5	1.52×10^8	2.87×10^9
1% / 3 min	2.82×10^5	5.8×10^7	1.92×10^9
1% / 5 min	2.53×10^5	6.5×10^7	1.94×10^9
1% / 7 min	2.5×10^5	6.8×10^7	1.88×10^9

APPENDIX TABLE 8

THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C		
	0 DAYS	5 DAYS	10 DAYS
	SPC/gm	SPC/gm	SPC/gm
Trial 1			
Controls	9.5×10^4	5.9×10^8	8.55×10^9
4% / 5 min	3.6×10^4	6.65×10^6	2.31×10^9
Trial 2			
Controls	1.27×10^6	1.31×10^8	6.35×10^9
4% / 5 min	1.35×10^5	3.5×10^6	1.57×10^9

APPENDIX TABLE 9

THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C		
	0 DAYS	5 DAYS	10 DAYS
	SPC/gm	SPC/gm	SPC/gm
Trial 3			
Controls	4.05×10^5	1.12×10^8	5.5×10^9
4% / 5 min	4.45×10^4	7.4×10^6	1.57×10^9
Trial 4			
Controls	4.45×10^6	2.11×10^8	4.35×10^9
4% / 5 min	1.07×10^5	7.15×10^6	1.21×10^9

APPENDIX TABLE 10

THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C		
	0 DAYS	5 DAYS	10 DAYS
	SPC/gm	SPC/gm	SPC/gm
Trial 1			
Controls	5.8×10^3	7.45×10^6	4.56×10^9
5% / 5 min	4.2×10^3	2.2×10^5	4.7×10^7
Trial 2			
Controls	6.75×10^3	1.92×10^7	5.3×10^9
5% / 5 min	4.05×10^3	1.9×10^5	1.32×10^8

APPENDIX TABLE 11

THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C		
	0 DAYS	5 DAYS	10 DAYS
	SPC/gm	SPC/gm	SPC/gm
Trial 3			
Controls	6.7×10^3	2.79×10^8	1.09×10^9
5% / 5 min	4.45×10^3	1.07×10^6	1.5×10^6
Trial 4			
Controls	1.1×10^4	1.5×10^7	1.79×10^9
5% / 5 min	6.25×10^3	2.6×10^5	2.9×10^7

APPENDIX TABLE 12

THE EFFECT OF POTASSIUM SORBATE ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C			
	0 DAYS	6 DAYS	10 DAYS	14 DAYS
Trial 1	SPC/gm	SPC/gm	SPC/gm	SPC/gm
Controls	1.46×10^5	3.22×10^7	2.0×10^8	2.8×10^9
9% / 5 min	1.20×10^5	3.5×10^4	1.0×10^4	1.0×10^8
Trial 2				
Controls	1.04×10^5	6.25×10^7	1.02×10^9	3.0×10^{10}
9% / 5 min	1.50×10^5	5.5×10^4	----NA*	----NA*
Trial 3				
Controls	9.75×10^4	5.55×10^7	6.9×10^9	3.0×10^{10}
9% / 5 min	6.85×10^4	3.5×10^4	2.3×10^5	1.0×10^7

* NA Not available due to spoiled sample.

APPENDIX TABLE 13

THE EFFECT OF POTASSIUM SORBATE IN COMBINATION WITH DISODIUM EDTA ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C			
	0 DAYS	6 DAYS	10 DAYS	14 DAYS
	SPC/gm	SPC/gm	SPC/gm	SPC/gm
Trial 1				
Controls	6.35×10^4	8.25×10^7	7.8×10^7	3.0×10^{10}
KSA-EDTA*	3.05×10^4	1.0×10^4	1.0×10^4	1.0×10^7
Trial 2				
Controls	1.25×10^5	1.01×10^8	2.7×10^7	3.0×10^7
KSA-EDTA*	6.04×10^4	4.5×10^4	1.0×10^4	1.0×10^7
Trial 3				
Controls	6.3×10^4	3.4×10^7	7.0×10^8	3.0×10^{10}
KSA-EDTA*	2.44×10^4	9.5×10^4	1.0×10^4	1.0×10^7

* KSA-EDTA 4% EDTA / 5 minutes followed by 9% potassium sorbate / 5 minutes.

APPENDIX TABLE 14

THE EFFECT OF DISODIUM EDTA ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3°C			
	0 DAYS	6 DAYS	10 DAYS	14 DAYS
	SPC/gm	SPC/gm	SPC/gm	SPC/gm
Trial 1				
Controls	1.12×10^5	3.75×10^7	1.74×10^9	3.0×10^{10}
4% $EDTANa_2$/5 min	6.1×10^4	4.65×10^6	6.0×10^8	1.05×10^8
Trial 2				
Controls	6.75×10^4	4.70×10^7	1.44×10^9	3.0×10^{10}
4% $EDTANa_2$/5 min	1.10×10^4	9.2×10^5	1.65×10^7	2.0×10^7

APPENDIX TABLE 15

THE EFFECT OF POTASSIUM SORBATE AT VARYING EXPOSURE TIMES ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS(8% potassium sorbate)	STORAGE DAYS AT 3°C			
	0 DAYS	6 DAYS	10 DAYS	14 DAYS
	SPC/gm	SPC/gm	SPC/gm	SPC/gm
Trial 1				
Controls	8.0×10^6	1.5×10^9	1.2×10^{10}	3.0×10^{10}
3 min	1.54×10^6	8.05×10^5	3.2×10^6	3.58×10^8
5 min	1.13×10^6	1.25×10^5	2.09×10^6	$<30. \times 10^5$*
7 min	1.95×10^5	4.3×10^5	5.5×10^4	$<30. \times 10^5$*

* Less than 30 colonies on a 10^5 dilution plate.

APPENDIX TABLE 16

THE EFFECT OF POTASSIUM SORBATE AT VARYING EXPOSURE TIMES ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS (8% potassium sorbate)	STORAGE DAYS AT 3°C			
	0 DAYS	6 DAYS	10 DAYS	14 DAYS
	SPC/gm	SPC/gm	SPC/gm	SPC/gm
Trial 2				
Controls	1.5×10^7	2.0×10^9	7.75×10^9	3.0×10^{10}
3 min	3.2×10^5	2.7×10^5	7.65×10^5	6.3×10^7
5 min	1.13×10^5	2.25×10^5	4.1×10^5	$< 30. \times 10^5$*
7 min	1.38×10^5	2.7×10^5	7.5×10^4	1.0×10^5

* Less than 30 colonies on a 10^5 dilution plate.

APPENDIX TABLE 17

THE EFFECT OF DISODIUM EDTA AT VARYING EXPOSURE TIMES ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS (8% EDTA)	STORAGE DAYS AT 3° C			
	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	SPC/gm.	SPC/gm.	SPC/gm.	SPC/gm.
CONTROLS	1.15×10^7	1.75×10^9	9.93×10^9	3.0×10^{10}
3 min	4.09×10^5	2.0×10^7	2.36×10^8	3.25×10^9
5 min	8.05×10^5	9.1×10^6	2.4×10^8	9.2×10^8
7 min	1.04×10^6	6.05×10^6	2.11×10^8	1.75×10^9

APPENDIX TABLE 18

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS (6% / 5 min)	STORAGE DAYS AT 3° C 0 DAYS	6 DAYS	10 DAYS	20 DAYS
Trial 1	SPC/gm.	SPC/gm.	SPC/gm.	SPC/gm.
CONTROLS	1.02×10^5	5.0×10^8	2.0×10^9	3.0×10^{10}
K-SA	5.75×10^5	9.45×10^5	2.2×10^6	3.71×10^7
EDTA	1.19×10^5	6.4×10^6	6.75×10^6	7.7×10^7
EDTA/ K-SA	4.45×10^4	7.5×10^4	3.4×10^4	$\leq 30. \times 10^5$

APPENDIX TABLE 19

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS (6% / 5 min)	STORAGE DAYS AT 3° C 0 DAYS	6 DAYS	10 DAYS	20 DAYS
	SPC/gm.	SPC/gm.	SPC/gm.	SPC/gm.
Trial 2				
CONTROLS	2.77×10^6	1.0×10^9	2.0×10^9	3.0×10^{10}
K-SA	1.19×10^5	9.0×10^4	1.62×10^6	3.06×10^8
EDTA	5.9×10^4	6.9×10^5	1.35×10^4	6.2×10^8
EDTA/ K-SA	1.58×10^4	2.5×10^4	1.5×10^4	$<30. \times 10^5$

APPENDIX TABLE 20

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS (8% / 5 min)	STORAGE DAYS AT 3° C			
	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	SPC/gm.	SPC/gm.	SPC/gm.	SPC/gm.
Trial 1				
CONTROLS	1.02×10^5	5.0×10^8	2.0×10^9	3.0×10^{10}
K-SA	4.3×10^4	1.5×10^4	1.25×10^5	7.0×10^6
EDTA	9.65×10^3	2.25×10^5	4.75×10^6	3.23×10^8
EDTA/ K-SA	1.01×10^4	6.0×10^4	1.0×10^5	$< 30. \times 10^5$

APPENDIX TABLE 21

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS (8% / 5 min)	STORAGE DAYS AT 3° C 0 DAYS	6 DAYS	10 DAYS	20 DAYS
	SPC/gm	SPC/gm	SPC/gm	SPC/gm
Trial 2				
CONTROLS	2.77×10^6	1.0×10^9	2.0×10^9	3.0×10^{10}
K-SA	2.05×10^5	1.7×10^5	2.0×10^5	5.0×10^6
EDTA	4.15×10^4	1.35×10^5	1.01×10^7	1.57×10^8
EDTA/ K-SA	1.43×10^4	6.6×10^4	1.0×10^5	3.0×10^5

APPENDIX TABLE 22

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS (10%/ 5 min)	STORAGE DAYS AT 3° C			
	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	SPC/gm	SPC/gm	SPC/gm	SPC/gm
Trial 1				
CONTROLS	1.02×10^5	5.0×10^8	2.0×10^9	3.0×10^{10}
K-SA	4.05×10^4	6.5×10^4	2.6×10^5	1.0×10^5
EDTA	2.56×10^4	5.3×10^5	5.15×10^5	7.9×10^7
EDTA/ K-SA	2.6×10^3	2.0×10^4	$< 30. \times 10^4$	$30. \times 10^4$

APPENDIX TABLE 23

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

TREATMENTS (10% / 5 min)	STORAGE DAYS AT 3° C 0 DAYS	6 DAYS	10 DAYS	20 DAYS
	SPC/gm	SPC/gm	SPC/gm	SPC/gm
Trial 2				
CONTROLS	2.77×10^{6}	1.0×10^{9}	2.0×10^{9}	3.0×10^{10}
K-SA	5.8×10^{4}	2.5×10^{4}	4.0×10^{4}	1.0×10^{5}
EDTA	1.25×10^{5}	4.25×10^{5}	1.43×10^{6}	$<30. \times 10^{5}$
EDTA/ K-SA	1.51×10^{4}	5.0×10^{3}	5.0×10^{3}	1.40×10^{8}

APPENDIX TABLE 24

THE EFFECT OF POTASSIUM SORBATE AT VARYING EXPOSURE TIMES

ON THE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3° C			
(8% K-SA)	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	P-SPC/gm	P-SPC/gm	P-SPC/gm	P-SPC/gm
Trial 1				
CONTROLS	1.6×10^5	2.5×10^8	8.0×10^9	3.0×10^{10}
3 min	5.8×10^5	7.3×10^5	6.05×10^6	2.89×10^8
5 min	5.08×10^5	1.11×10^5	1.89×10^6	$< 30. \times 10^5$
7 min	1.85×10^5	2.9×10^5	2.0×10^4	3.0×10^5

APPENDIX TABLE 25

THE EFFECT OF POTASSIUM SORBATE AT VARYING EXPOSURE TIMES

ON THE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

TREATMENTS (8% K-SA)	STORAGE DAYS AT 3° C 0 DAYS	6 DAYS	10 DAYS	20 DAYS
	P-SPC/gm	P-SPC/gm	P-SPC/gm	P-SPC/gm
Trial 2				
CONTROLS	3.0×10^{6}	3.0×10^{8}	8.0×10^{9}	3.0×10^{10}
3 min	6.0×10^{5}	5.55×10^{4}	5.6×10^{5}	5.3×10^{7}
5 min	3.9×10^{4}	1.56×10^{5}	5.75×10^{5}	1.0×10^{5}
7 min	1.14×10^{5}	2.75×10^{5}	2.0×10^{4}	1.0×10^{5}

APPENDIX TABLE 26

THE EFFECT OF DISODIUM EDTA AT VARYING EXPOSURE TIMES

ON THE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3° C			
(8% EDTA Na_2)	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	P-SPC/gm	P-SPC/gm	P-SPC/gm	P-SPC/gm
CONTROLS	1.58×10^6	2.75×10^8	8.0×10^9	3.0×10^{10}
3 min	1.02×10^5	1.85×10^7	2.47×10^8	3.64×10^9
5 min	7.0×10^5	9.26×10^6	2.49×10^8	1.61×10^9
7 min	6.0×10^5	6.6×10^6	2.09×10^8	2.27×10^9

APPENDIX TABLE 27

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION

ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3° C			
(6% / 5 min)	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	P-SPC/gm	P-SPC/gm	P-SPC/gm	P-SPC/gm
Trial 1				
CONTROLS	2.0×10^{6}	3.0×10^{8}	2.0×10^{9}	3.0×10^{10}
K-SA	1.61×10^{5}	1.27×10^{6}	2.0×10^{6}	1.12×10^{7}
EDTA	2.85×10^{4}	4.1×10^{6}	8.75×10^{6}	8.0×10^{7}
EDTA/ K-SA	8.15×10^{2}	7.0×10^{3}	5.0×10^{3}	$< 30. \times 10^{5}$

APPENDIX TABLE 28

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

TREATMENTS (6% / 5 min)	STORAGE DAYS AT 3° C 0 DAYS	6 DAYS	10 DAYS	20 DAYS
Trial 2	P-SPC/gm	P-SPC/gm	P-SPC/gm	P-SPC/gm
CONTROLS	8.0×10^5	5.0×10^8	2.0×10^9	3.0×10^{10}
K-SA	7.75×10^4	9.8×10^4	1.52×10^6	3.29×10^8
EDTA	5.85×10^3	6.0×10^5	1.35×10^4	6.5×10^8
EDTA/ K-SA	2.10×10^3	2.95×10^4	1.0×10^3	1.0×10^5

APPENDIX TABLE 29

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION

ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3° C			
(8% / 5 min)	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	P-SPC/gm	P-SPC/gm	P-SPC/gm	P-SPC/gm
Trial 1				
CONTROLS	2.0×10^{6}	3.0×10^{8}	2.0×10^{9}	3.0×10^{10}
K-SA	1.14×10^{4}	7.0×10^{3}	4.1×10^{4}	5.0×10^{5}
EDTA	4.7×10^{3}	2.47×10^{5}	5.0×10^{6}	3.89×10^{8}
EDTA/ K-SA	2.5×10^{2}	5.75×10^{4}	1.5×10^{3}	$< 30. \times 10^{5}$

APPENDIX TABLE 30

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION

ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3° C			
(8% / 5 min)	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	P-SPC/gm	P-SPC/gm	P-SPC/gm	P-SPC/gm
Trial 2				
CONTROLS	8.0×10^5	5.0×10^8	2.0×10^9	3.0×10^{10}
K-SA	5.35×10^4	4.05×10^4	1.05×10^5	7.0×10^5
EDTA	1.11×10^4	1.43×10^5	1.07×10^7	1.30×10^8
EDTA/ K-SA	8.0×10^1	5.0×10^4	1.6×10^4	7.0×10^5

APPENDIX TABLE 31

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION

ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3° C			
(10% / 5 min)	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	P-SPC/gm	P-SPC/gm	P-SPC/gm	P-SPC/gm
Trial 1				
CONTROLS	2.0×10^{6}	3.0×10^{8}	2.0×10^{9}	3.0×10^{10}
K-SA	3.18×10^{4}	6.4×10^{4}	1.86×10^{5}	1.0×10^{5}
EDTA	1.39×10^{4}	5.7×10^{5}	6.6×10^{5}	1.15×10^{8}
EDTA/ K-SA	4.9×10^{2}	$< 30. \times 10^{3}$	$< 30. \times 10^{3}$	$< 30. \times 10^{5}$

APPENDIX TABLE 32

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

TREATMENTS	STORAGE DAYS AT 3° C			
(10% / 5 min)	0 DAYS	6 DAYS	10 DAYS	20 DAYS
	P-SPC/gm	P-SPC/gm	P-SPC/gm	P-SPC/gm
Trial 2				
CONTROLS	8.0×10^5	5.0×10^8	2.0×10^9	3.0×10^{10}
K-SA	2.5×10^4	1.35×10^4	1.75×10^4	1.0×10^5
EDTA	5.15×10^4	4.2×10^5	1.31×10^6	3.0×10^6
EDTA/ K-SA	1.27×10^3	1.15×10^4	5.0×10^2	9.5×10^7

APPENDIX TABLE 33

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

TABLE NUMBER	LEVELS OF SIGNIFICANCE 90%	95%	T (TEST STATISTIC)	LEVEL OF SIGNIFICANCE
7	1.886	2.920	2.865	90 - 95%
8 & 9	1.886	2.920	3.741	>95%
10 & 11	1.886	2.920	2.265	90 - 95%
12	1.638	2.353	2.839	95%
13	1.638	2.353	3.241	95%
14	1.638	2.353	2.457	95%

Data is from Appendix Tables 7 to 14

APPENDIX TABLE 34

THE EFFECT OF POTASSIUM SORBATE AT VARYING EXPOSURE TIMES ON THE VIABLE PSYCHROTROPHIC BACTERIA

ANOVA

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F -VALUE
TREATMENTS	3	40.10410	13.6347	14.670056 **
ERROR	12	11.15306	0.929423	F_{cr} = 5.95
TOTALS	15	52.05718		

Data is from Appendix Tables 15 and 16.

F values * significant
** very significant

APPENDIX TABLE 35

THE EFFECT OF DISODIUM EDTA AT VARYING EXPOSURE TIMES ON THE VIABLE PSYCHROTROPHIC BACTERIA

A N O V A

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
TREATMENTS	3	7.53587	2.51195	1.1065977
ERRORS	12	27.23979	2.269983	
TOTAL	15	34.775669638		

Data is from Appendix Table 17.

APPENDIX TABLE 36

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

A N O V A

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
TREATMENTS	3	33.12655	11.04218	6.2153**
ERROR	12	21.31944	1.77662	
TOTAL	15	54.44598		

Data is from Appendix Tables 18 and 19.

F values * significant
** very significant

APPENDIX TABLE 37

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

A N O V A

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
TREATMENTS	3	54.95253	11.61428	6.93056**
ERROR	12	34.84284	1.67581	
TOTAL	15	20.1096		

Data is from Appendix Tables 20 and 21.

F values * significant
** very significant

APPENDIX TABLE 38

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PSYCHROTROPHIC BACTERIA

A N O V A

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
TREATMENTS	3	63.74404	12.80258	6.05172**
ERROR	12	38.40774	2.115525	
TOTAL	15	25.3863		

Data is from Appendix Tables 22 and 23.

F values * significant
** very significant

APPENDIX TABLE 39

THE EFFECT OF POTASSIUM SORBATE AT VARYING EXPOSURE TIMES

ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

A N O V A

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
TREATMENTS	3	33.383829	11.12994	7.56116**
ERROR	12	17.66069	1.47172	
TOTAL	15	51.04452		

Data is fromAppendix Tables 24 and 25.

F values ** very significant

APPENDIX TABLE 40

THE EFFECT OF DISODIUM EDTA AT VARYING EXPOSURE TIMES ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

A N O V A

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
TREATMENTS	3	4.167974	1.389313	0.758555
ERROR	12	36.357185	3.024765	
TOTAL	15	40.52516		

Data is from Appendix Table 26

APPENDIX TABLE 41

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

A N O V A

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
TREATMENTS	3	43.41689	14.47230	6.45212**
ERROR	12	26.91645	2.24303	
TOTAL	15	70.33333		

Data is from Appendix Tables 27 and 28

F values ** very significant

APPENDIX TABLE 42

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

A N O V A

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
TREATMENTS	3	46.797143	15.59905	6.46787**
ERROR	12	28.94131	2.41178	
TOTAL	15	75.73845		

Data is from Appendix Tables 29 and 30.

F values ** very significant

APPENDIX TABLE 43

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON THE VIABLE PENICILLIN RESISTANT PSYCHROTROPHIC BACTERIA

A N O V A

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
TREATMENTS	3	45.21704	15.07236	5.62899**
ERROR	12	32.13165	2.67764	
TOTAL	15	77.34875		

Data is from Appendix Tables 31 and 32.

F values ** very significant

APPENDIX TABLE 44

THE EFFECT OF EDTA ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
4% EDTA Na_2/ 5 min					
Trial 1					
CONTROLS	0 0 0	0 0 1	2 2 2	3 3 3	4 4 4
TREATMENTS	0 0 0	0 0 0	2 1 0	2 1 3	3 3 3
Trial 2					
CONTROLS	0 0 0	0 0 0	1 1 1	4 4 4	4 4 4
TREATMENTS	0 0 0	0 0 0	0 0 0	1 1 1	3 3 3

APPENDIX TABLE 45

THE EFFECT OF EDTA ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
(4% EDTA Na_2Ca/ 5 min)	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
Trial 1					
CONTROLS	0 0 0	0 0 0	1 1 1	3 4 2	4 4 4
TREATMENTS	0 0 0	0 0 0	0 0 0	1 1 1	3 3 3
Trial 2					
CONTROLS	0 0 0	1 2 0	1 1 1	4 4 4	4 4 4
TREATMENTS	0 0 0	0 0 0	1 1 1	2 2 2	4 4 4

APPENDIX TABLE 46

THE EFFECT OF POTASSIUM SORBATE ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
(9% K-SA/ 5 min)					
Trial 1					
CONTROLS	0 0 0	1 1 1	2 1 3	4 4 4	4 4 4
TREATMENTS	0 0 0	0 0 0	0 0 0	0 0 0	1 2 0
Trial 2					
CONTROLS	0 0 0	0 0 0	1 1 1	4 4 4	4 4 4
TREATMENTS	0 0 0	0 0 0	0 0 0	1 1 1	1 2 1
Trial 3					
CONTROLS	0 0 0	1 1 1	1 1 1	3 4 3	4 4 4
TREATMENTS	0 0 0	0 0 0	0 0 0	1 0 1	0 1 0

APPENDIX TABLE 47

THE EFFECT OF POTASSIUM SORBATE IN COMBINATION WITH DISODIUM EDTA ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
(9% K-SA + 4% EDTA Na_2)					
Trial 1					
CONTROLS	0 0 0	1 1 1	1 1 1	4 4 4	4 4 4
TREATMENTS	0 0 0	0 0 0	0 0 0	2 3 2	0 0 0
Trial 2					
CONTROLS	0 0 0	1 1 1	1 2 0	4 4 4	4 4 4
TREATMENTS	0 0 0	0 0 0	0 0 0	1 1 1	1 1 2
Trial 3					
CONTROLS	0 0 0	0 0 0	1 1 1	4 4 4	4 4 4
TREATMENTS	0 0 0	0 0 0	0 0 0	1 1 1	1 1 1

APPENDIX TABLE 48

THE EFFECT OF POTASSIUM SORBATE AT VARYING EXPOSURE TIMES ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
(8% K-SA)	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
Trial 1					
CONTROLS	0 0 0	1 2 1	3 4 3	4 4 4	4 4 4+++
3 min	0 0 0	0 1 0	1 1 1	1 1 1	0 1 1
5 min	0 0 0	1 0 2	2 1 3	1 2 0	0 1 1
7 min	0 0 0	0 1 0	1 1 1	0 0 0	0 0 1

APPENDIX TABLE 49

THE EFFECT OF POTASSIUM SORBATE AT VARYING EXPOSURE TIMES ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
(8% K-SA)					
Trial 2					
CONTROLS	0 0 0	2 2 2	4 4 4	4 4 4	4 4 4+++
3 min	0 0 0	1 1 1	1 2 1	1 1 2	0 0 1
5 min	0 0 0	0 1 1	1 1 1	1 1 0	0 1 1
7 min	0 0 0	1 0 0	1 1 0	1 1 1	1 0 0

APPENDIX TABLE 50

THE EFFECT OF DISODIUM EDTA AT VARYING EXPOSURE TIMES ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
(8% EDTA Na_2)	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
CONTROLS	0 0 0	2 1 2	4 4 3	4 4 4	4 4 4
3 min	0 0 0	0 1 0	0 1 1	1 1 2	2 2 2
5 min	0 0 0	0 0 0	0 1 0	1 1 1	1 3 2
7 min	0 0 0	0 1 0	1 1 0	1 1 1	1 2 2

APPENDIX TABLE 51

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
(6% / 5 min)	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
Trial 1					
CONTROLS	0 0 1	1 0 1	1 1 2	4 4 4	4 4 4+++
K-SA	0 0 0	0 0 0	0 0 0	0 1 1	1 1 0
EDTA	0 0 0	0 0 0	0 0 0	0 0 0	1 3 2
EDTA/ K-SA	0 0 0	0 0 1	1 1 1	0 0 0	2 0 0

+++ This rating went far over the 4 point rating scale.

APPENDIX TABLE 52

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
(6% / 5 min)					
Trial 2					
CONTROLS	0 0 0	0 1 0	2 1 1	4 4 4	4 4 4+++
K-SA	0 0 0	0 0 0	0 0 1	1 0 1	2 0 0
EDTA	0 0 0	0 0 0	0 0 0	0 0 0	1 0 0
EDTA/ K-SA	0 0 0	0 0 0	0 0 0	2 2 3	2 0 0

+++ This rating went far over the 4 point rating scale.

APPENDIX TABLE 53

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
(8% / 5 min)					
Trial 1					
CONTROLS	0 0 1	1 0 1	1 1 2	4 4 4	4 4 4+++
K-SA	0 0 0	0 0 0	0 0 0	0 1 1	1 0 0
EDTA	0 0 0	0 0 0	0 0 0	0 0 0	0 3 2
EDTA/ K-SA	0 0 0	0 0 1	0 1 1	0 0 0	1 1 3

+++ This rating went far over the 4 point rating scale.

APPENDIX TABLE 54

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
(8% / 5 min)					
Trial 2					
CONTROLS	0 0 0	0 1 0	2 1 1	4 4 4	4 4 4+++
K-SA	0 0 0	0 1 0	0 1 0	0 1 1	1 0 0
EDTA	0 0 0	0 1 0	1 1 1	1 2 3	0 0 0
EDTA/ K-SA	0 0 0	0 0 0	0 1 0	1 0 1	1 0 0

+++ This rating went far over the 4 point rating scale.

APPENDIX TABLE 55

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
(10% / 5 min)					
Trial 1					
CONTROLS	0 0 0	0 1 0	2 1 1	4 4 4	4 4 4+++
K-SA	0 0 0	1 0 0	1 1 0	1 1 1	2 1 0
EDTA	0 0 0	0 0 0	0 0 0	2 3 2	2 0 2
EDTA/ K-SA	0 0 0	0 0 0	0 0 0	1 1 0	1 0 0

+++ This rating went far over the 4 point rating scale.

APPENDIX TABLE 56

THE EFFECT OF POTASSIUM SORBATE, DISODIUM EDTA, AND THEIR COMBINATION ON ODOUR RATINGS

TREATMENTS	STORAGE DAYS AT 3° C				
	0 DAYS	3 DAYS	6 DAYS	10 DAYS	20 DAYS
	ODOUR RATINGS (3 INDEPENDENT JUDGES)				
(10% / 5 min)					
Trial 2					
CONTROLS	0 0 0	0 1 0	2 1 1	4 4 4	4 4 4+++
K-SA	0 0 0	1 0 0	1 1 0	1 1 1	2 1 0
EDTA	0 0 0	0 0 0	0 0 0	2 3 2	2 0 2
EDTA/ K-SA	0 0 0	0 0 0	0 0 0	1 1 0	1 0 0

+++ This rating went far over the 4 point rating scale.