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Title PRESERVATION OF DOVER SOLE BY LOW-DOSE RADIATION AND ANTIMICROBIAL AGENTS

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The storage life at 43° F of ground dover sole fillets (Microstomas pacificus) was determined microbiologically after treatment with ionizing radiation and antimicrobial agents. Sodium benzoate, potassium sorbate and the sodium salts of the methyl and propyl esters of parahydroxybenzoic acid (MPB and PPB) all approximately doubled the refrigerated storage life of irradiated dover sole. The dose levels employed were 0.1, 0.3, and 0.5 Mrad. The concentration of the agents used in each case was 0.1 percent.

The normal spoilage of dover sole is caused predominately by the outgrowth of pseudomonads. After irradiation, however, the spoilage is due to the outgrowth of Achromobacter and certain Gram positive organisms. The spoilage pattern was not changed by the antimicrobial agents tested except at the higher dose level (0.5 Mrad), at which the spoilage was caused by yeasts.

The additives had no effect on the growth rate of the microorganisms of dover sole. They merely prolonged the length of microbial dormancy that followed radiation exposure. Possible mechanisms involved in the combined effect of antimicrobial agents and irradiation are discussed.

PRESERVATION OF DOVER SOLE BY LOW-DOSE RADIATION AND ANTIMICROBIAL AGENTS

by

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INTRODUCTION

Low-dose ionizing radiation has exhibited promise as a method of extending the refrigerated storage life of perishable foods. At the same time, the pasteurization process is expected to eliminate pathogenic microorganisms that may be present.

For an increased margin of safety it would be desirable to use a sufficiently high level of radiation for this process. At the higher dosages, the possibility of the survival of pathogenic microorganisms would be lessened. However, higher levels of radiation sometimes lead to the development of off-flavors and off-odors. Means have been sought to increase the effectiveness of radiation without increasing the undesirable side effects.

It is for this reason the research discussed in this thesis was undertaken. Various antimicrobial agents were investigated for the possibility of potentiating the effectiveness of radiation. All of the agents investigated have been approved by the United States Food and Drug Administration for use in food in the concentrations employed (43, p. 203-207). Sodium benzoate is commonly used commercially as a dip for fishery products. The methyl and propyl esters of para-hydroxybenzoic acid are used in a variety of products including sausages, fresh vegetables and syrups. Potassium sorbate is not usually

used with meat products, but is frequently employed as a fungistat in cheeses, pickles, fruit juices and many other types of foods.

LITERATURE REVIEW

Radiation Preservation

Ionizing radiations, so called because of their ability to eject planetary electrons from atoms and molecules, possess several novel attributes, including their high degree of penetrating power and their great energy. These properties provide widespread applications in biology, medicine and agriculture, from destroying a cancerous growth in a man's body to preserving his food. Platzman (29, p. 74-75) explained that the varieties of ionizing radiation produce in different ways the same sort of ultimate effects on matter. The interaction of each type of radiation with matter depends upon the mass and the charge of the radiation as well as upon its energy.

There are two dose ranges used in radiation preservation of food described by the Army Quartermaster Corps (47, p. 1): (1) High dose level, which is greater than one megarad (Mrad) and usually in the range of 2.0 - 4.5 Mrad. This is termed sterilization because its purpose is to kill all the microorganisms present, thereby providing a capability for long term storage without refrigeration. (2) Low dose level, which is one Mrad or less. This is termed pasteurization because it destroys or inhibits certain undesirable bacteria in food in about the same degree as conventional pasteurizing methods for milk. Radiation pasteurization has the capability of extending the

useful life of perishable food products under refrigeration. The latter type of process will be dealt with in this thesis. The Army Quartermaster Corps has concentrated mainly on the sterilization process in an attempt to develop a field-ration product. Many investigators, including Rhodes (31, p. 5-10) and Luck (20, p. 535-549) believe that the radiation sterilization of foods will not be feasible commercially, due to the flavor changes which often occur. Pasteurization by radiation, because of the lesser changes in flavor, is believed to have better commercial potential. Since 1959, the United States Atomic Energy Commission has been sponsoring much of the research in this area in the United States.

Safety of Radiation Preserved Foods

The wholesomeness and safety of irradiated foods has been the subject of many investigations. The results of these experiments indicated that a wide variety of foods treated at various doses between 0.01 and 6 Mrad were all entirely suitable for inclusion in the human diet, according to Rhodes (31, p. 18). At a dose of 0.3 Mrad the nutritive value of a high protein food such as fish was not measureably affected and no toxic or carcinogenic factors were produced.

In tests performed by Read, et al. (30, p. 153-173), four successive generations of rats were fed with a composite diet prepared from nine components, each of which had been previously irradiated

with 5. 58 Mrad of gamma radiation. Reproductive performance, life span and incidence of disease, including occurrence of neoplasms, were not affected. Metabolizable energy value of the diet was not affected by the irradiation. Activities of liver xanthine oxidase, succinic dehydrogenase, hypotonic endogenous respiration, alkaline phosphatase and serum alkaline phosphatase were unaffected in these rats.

Results from tests devised by the staff of the Office of the Surgeon General of the United States and the National Academy of Science in cooperation with the Food and Drug Administration may be summarized from Desrosier and Rosenstock (11, p. 303-305) as follows: No toxic substances were found to develop from any source; the nutritional adequacy of the proteins was acceptable; vitamins might have undergone various degrees of destruction, comparable with that encountered in other methods of preservation; no significant amounts of induced radioactivity were found.

Applications of Radiation Pasteurization

The radiation pasteurization treatment is applicable to a great many foods. One of its great advantages, and perhaps the area where it will eventually find its greatest use commercially, is the possibility of extending the storage life of fresh commodities, without altering their freshlike qualities. Fresh vegetables, fruit, meats and seafood products would find a larger market.

Fruits showing promise of benefit through low dose radiation include strawberries, grapes, peaches, tomatoes, and citrus fruits, according to reports given to the Army Quartermaster Corps (47, p. 64). General trends indicated that from 0.2 - 0.8 Mrad materially increased the shelflife by two to ten times under various storage conditions. This preservation process destroys surface flora and inhibits the progress of post-harvest diseases in vegetables. Used in conjunction with controlled temperature and humidity, this process offered a means of extending the holding times substantially. Potatoes particularly showed promise as a product for low-dose irradiation treatment. Such treatment would not only inhibit rot, but would control sprouting. Mullins and Burr (26, p. 178-179) reported that the same treatment was also beneficial in controlling rot and sprouting in onions.

Among meat products, pork was successfully processed by radiation. Researchers under the Army Quartermaster Corps (47, p. 7-13) found that irradiation of pork could control the trichina larvae. Organoleptically, pork generally responded more favorably than beef; however, fresh ground beef was fairly successful. Poultry received acceptable ratings on a taste panel, although chicken irradiated above 0.1 Mrad could easily be distinguished organoleptically from the unirradiated product.

Seafood was also found to be a feasible product for radiation

pasteurization. Radiation treatment of 0. 1 - 0. 8 Mrad would successfully inhibit microbial spoilage in many types of seafoods. Masurovsky, Goldblith, and Nickerson (22, p. 222-226) reported that haddock fillets could be kept in a microbiologically satisfactory state from one week to three months with doses from 0. 05 to 0. 8 Mrad of Co-60 gamma radiation. These investigators also found that shucked soft-shelled clams could be maintained for significantly longer periods in refrigerated storage than haddock fillets when the same radiation doses were applied to each product. The extended shelf-life of clams was confirmed by Connors and Steinberg (10, p. 1059-1060). They found the refrigerated storage time to be at least 30 days at doses of 0. 55 Mrad and that these clam meats were still organoleptically acceptable.

Organoleptic, chemical and microbiological tests performed by Scholz, et al. (35, p. 118-120) indicated that the storage life of crabmeat irradiated at 0. 25 Mrad was extended to three to four weeks, compared with one week for unirradiated samples. A dose of 0. 50 Mrad extended the shelf life to five weeks. Precooked shrimp irradiated at 0. 50 and 0. 75 Mrad were not spoiled even after an 18 week storage period.

Recent work at the Bureau of Commercial Fisheries, Seattle, Washington (24, p. 158-142) indicated that king crab meat packed in cans, irradiated at 0. 1 - 0. 6 Mrad and stored at 33° and 42° F could

be kept from four to six weeks, compared to one week for the control. Air-packed skinless pollock and ocean perch fillets treated with 0. 15 and 0. 25 Mrad, respectively, also benefitted by the low dose irradiation treatment according to Ronsivalli and Slavin (33, p. 20-27). The storage life was extended by more than one month at 33-35° F.

Problems in Radiation Pasteurization

One of the greatest problems in the radiation pasteurization of foods is the elimination of the undesirable side effects. Such side effects include flavor, odor, texture, and color changes. Luck (20, p. 535-549) stated that the changes caused by ionizing radiation depend upon dose, molecular weight and ionization rate. Therefore, in many cases, doses greater than 0. 05-0. 1 Mrad bring about changes of odor, flavor and structure. The extent of these changes depends upon the nature of the food. According to Rhodes, Sharp, and Ingram, (31, p. 221) one Mrad gives about 1-10 mmole of active radicals or ions per kilogram of irradiated tissue. This amount is comparable to the concentrations of individual minor components of meat. Radiation-sensitive substances, e. g. glutathione or sulphur-containing amino acids, are therefore extensively damaged, these particular compounds producing mercaptans which contribute largely to "irradiation odor". Carbonylic compounds are also formed from the side chains of other amino acids.

Tappel and co-workers (42, p. 20) investigated various marine products as to flavor, color and vitamin changes after irradiation. Among the products which underwent changes after irradiation was raw shrimp treated with 0. 5 Mrad. It developed a slight iodine-like quality and acquired a bleached appearance when cooked, suggesting some loss of the carotenoid pigments. Sole irradiated at 0. 5 Mrad produced some off-odors, although color and taste appeared unaffected. Salmon suffered the greatest loss in sensory quality. At 0. 5 Mrad the odor was described as slightly off, and severe bleaching was noted.

Shewan and Liston (36, p. 379) reported that a taste panel found the flavor of irradiated cod "very bitter, burnt, rubbery" and "inedible" when the dosage was 1. 0 Mrad; at 0. 5 Mrad the flavor was described as "bitter, burnt milk" and "just edible"; at 0. 25 Mrad, "some sweetness, very slightly burnt" and "edible". This was compared to the control which was described as "neutral, slightly sweet" flavored and "edible".

After irradiation, yellow, rancid spots sometimes appeared on fishery products, according to Stout (41, p. 38). It was assumed that these occurred because of rapid lipid oxidation, but no experimental substantiation has been made to prove this contention. Lerke, Farber and Huber (19, p. 15) found that in the case of medium-fatty fish, such as the rock-fish species, and in the case of the definitely fatty fish, such as salmon and tuna, fat spoilage during storage became a

complicating factor in addition to protein spoilage. They indicated that the use of an antioxidant greatly reduced lipid oxidation.

Radiation resistant microorganisms may present a problem, particularly if these organisms include pathogens. Morgan and Reed (25, p. 365) found that under arbitrarily standardized conditions, spores of Clostridium botulinum were more resistant to gamma radiation than spores of other food spoilage organisms investigated.

Schmidt and co-workers (34, p. 88) indicated that, "Any food product designed for human consumption and of such nature that it is capable of supporting the growth of C. botulinum, must be so processed and stored to assure public-health safety under the assumption that such contamination may be present." These workers found that the radiation resistance of spores of Type E C. botulinum is of such an order that radiation dose levels applied for radiation pasteurization are insufficient to destroy even moderate levels of Type E spore contamination. Furthermore, it was shown that small numbers of survivors of irradiated Type E spores can germinate and produce toxic outgrowth at 43° and 49° F. The time required is well within the estimates of the refrigerated storage life of some radiation pasteurized food products.

Schmidt, et al. (34, p. 89) further stated, "It is probable that today the successful use of refrigeration in the limited prolongation of the storage life of perishable food products is primarily due to the

fact that the normal indigenous flora, in great part psychrophilic in nature, produces very obvious organoleptic defects long before a public-health hazard can occur. The destruction of this flora by radiation or any other method for the purpose of extending refrigerated storage life removes an active agency in the assurance of public-health safety and suggests the necessity for the application of much more stringent control of the temperatures of refrigerated storage and distribution of such products than has hitherto been required."

Thatcher (44, p. 51-58) also discussed some of the public health aspects of radiation pasteurization. He indicated that irradiation may render more common some of the mutations encountered infrequently in nature. While within an irradiated population, many mutations would be lethal and the degree of change for a given parameter tends to be small and more frequently negative than positive nevertheless, a significant proportion would show a large order of increase. An increase in both degree of change and frequency could clearly be of concern if applicable to a factor endangering health, such as virulence, toxigenicity or antibiotic-resistance.

Another problem discussed by Thatcher is that of metabolic changes in foodborne organisms by modification of the biochemical changes brought about in food which could change spoilage characteristics and possibly give rise, for a specific food, to forms of spoilage not commonly encountered. This would disturb the ecology of the

total microbial population within the irradiated food with the final nature of microbial propagation somewhat unpredictable. He feared this may favor the selection of pathogens, such as staphylococci and salmonellae, which are somewhat more resistant than coliforms and other common spoilage bacteria. Yeasts and filamentous fungi are, in general, more resistant than vegetative cells of bacilli. Hence, a radiation pasteurized food would not only lack the measure of safety provided by Escherichia coli as an indicator of contamination, but the specificity of resistance would favor selection of food-poisoning and mycotic pathogens. There is another indication that radiation pasteurization may selectively spare viruses, according to Baron and Jensen (2, p. 677).

Thatcher (44, p. 54) stated that there are two practical propositions in the radiation pasteurization of foods: (1) it is necessary to protect the food from exposure to a post-irradiation environment conducive to selective multiplication of a mutant by providing refrigeration, freezing, dehydration or equivalent means of restricting growth; (2) a high proportion of the microbial population of a food should be destroyed by the radiation process, because a small number of survivors further minimizes the probability of finding undesirable mutants among these survivors.

Methods of Improving the Radiation Pasteurization Process

There are several ways of accomplishing the second of Thatcher's proposals. One suggestion, by Novak (28, p. 23) is that only the highest quality of fish be utilized for this process. This would leave fewer survivors, due to the lower initial load. Novak stated, "Quality of shrimp [or any food] is not improved by radiation pasteurization, and it should be understood that only products of high initial quality can be preserved successfully."

Another consideration would be to increase the dosage to the maximum. This may lead to organoleptic problems. Goldblith (13, p. 158) suggested several methods for reducing side effects in foodstuffs exposed to ionizing radiations. These included reduction of temperature to immobilize free radicals; reduction of oxygen tension to reduce the numbers of oxidative free radicals to activated molecules; addition of free radical scavengers which provide competition for free radicals; concurrent radiation distillation for removal of volatile off-flavor, off-odor precursors; and reduction of dose. To achieve the reduction of dose level without a reduction of preserving effectiveness, he suggested that the radiation pasteurization process be combined with other processes. Those which he mentioned were complemental use of ionizing energy and thermal or sonic energy, and the use of bacterial sensitizing compounds, spore germinating agents, etc.

Pre-cooking and post-cooking were also discussed by Thornley and Ingram (45, p. 45-46). The vegetative cells can be eliminated by these processes; the spores would also probably be reduced but not eliminated. Irradiation was shown to sensitize bacterial spores to subsequent heat treatment by Kan, Goldblith, and Proctor (16, p. 513). Curing is also mentioned by Thornley and Ingram (45, p. 46) as a possible combination treatment with irradiation. The curing ingredients could eliminate some of the pathogenic microorganisms provided that the use of radiation was not made an excuse for reducing the curing treatment. There is a possibility that irradiated spores may prove even less likely to germinate subsequently in cured meat than heated spores at equivalent levels of survival.

The above procedures would alter the fresh quality of the product. It is for this reason that investigations have been conducted on various types of food additives to improve the efficiency of radiation. One of the true sensitizers being investigated is vitamin K_5 and its analogs. Silverman, et al. (37, p. 687-691) found that spores of Bacillus subtilis and B. stearothermophilus irradiated in nitrogen were killed in greater numbers in the presence of vitamin K_5 and two of its analogs than when irradiated without the chemicals. These chemicals, however, did not sensitize bacteria in orange juice or in milk.

The tetracycline antibiotics were found useful in extending the storage life of irradiated fishery products. Shewan and Liston (36,

p. 377-383) used chlortetracycline in combination with irradiation to preserve cod fillets. They reported that antibiotics appeared to prevent the recovery of some of the bacteria which survived the physical damage caused by irradiation. These bacteria, however, eventually returned to normal growth.

Lerke, et al. (19, p. 145) reported that the combined use of chlortetracycline and beta radiation was beneficial in retarding spoilage of flatfish and rock fish fillets, salmon steaks, precooked tuna loins, cooked crabmeat and shrimp.

The tetracycline antibiotics were found effective in the inhibition of spoilage of irradiated meats by Cain, Anderson and Malaspina (9, p. 583). The supplementary effect of the antibiotic was greater when the samples were irradiated at 0. 1 Mrad than at 0. 3 Mrad. The sample which had the best color and odor throughout the storage period was that which was treated with 0. 1 Mrad with four parts per million oxytetracycline. They also reported the loss of antibiotic activity as a result of radiation exposure.

MATERIALS AND METHODS

Seafood

Dover sole (Microstomas pacificus) fillets were obtained through the Seafoods Laboratory of Food Science and Technology Department, Oregon State University located at Astoria, Oregon from fish processing plants in the Astoria area. The fillets were chilled and shipped on ice to Corvallis by airplane on the day of filleting.

When the fillets were needed only as the source of microorganisms, fresh-frozen samples were used.

Preparation of the Sample

The fillets were ground in a sterile meat grinder by hand and mixed thoroughly in sterile jars by vigorous agitation. This grinding and mixing eliminated the sample to sample variation experienced by MacLean and Welander (21, p. 251-252). The preservative, if used before irradiation, was added at this point to a weighed amount of ground fish flesh to give a final concentration of 0.1%. The ground fish was then weighed in covered sterile Petri dishes and packed in small screw-cap vials. Each vial contained 10 grams of sample. The samples were prepared aseptically using alcohol-flamed spatulas.

Irradiation

Two different radiation sources were employed: The radiation source of the Department of Interior, Bureau of Mines, Albany, Oregon and the radiation source located in the Radiation Center, Oregon State University. Both are Cobalt-60 gamma sources. The former source had a dose rate in air of 8.8×10^5 rad per hour; the latter, 8.09×10^5 rad per hour.

The sample vials were transported to and from the radiation sources on ice.

Storage

A uniform storage temperature of 43° F was employed for this study. This temperature was selected to simulate average household refrigerator conditions.

Microbiological Examination

Total Counts

Two vials from each treatment were taken out of storage at appropriate intervals, and the whole content of a vial was emptied into 90 ml of 0.2% Bacto - Peptone - water dilution blanks. This was equal to a 1/10 dilution. The diluent also contained 15 grams glass beads to facilitate the breaking up of the tissue. Series of 1/10

dilutions were subsequently made using 0. 2% peptone-water diluents. This diluent was adapted from Sinnhuber and Lee (38, p. 10), who reported that more microorganisms could be recovered from dover sole when 0. 2% peptone-water was used then from distilled water, phosphate buffer or 0. 5% peptone.

One-tenth ml portions of the proper dilutions were pipetted on solidified tryptone-glucose-yeast extract-NaCl (TN) agar in triplicates and evenly spread on the entire agar surface with sterile L-shaped glass rods.

The plates were inverted and incubated at 30° C for 24-48 hours or until no new colonies appeared. The total microbial counts were obtained from the average of triplicates. The incubation temperature of 30° C was selected because this temperature will not inhibit most psychrophilic growth and will permit the mesophilic microorganisms to grow, according to Ingraham and Stokes (15, p. 100-103).

Microbial Flora Analysis

The aerobic facultative microorganisms were identified according to the replica plating method described by Sinnhuber and Lee (38, p. 7, 52-53). Master plates were prepared by picking colonies from the initial isolation plates onto thick TN plates using sterile toothpicks. Twenty-one spots were marked at appropriate intervals on the bottom of the Petri dish and transfer was made onto corresponding locations

on the agar surface. Each location was assigned a number from one to twenty-one. The colonies on the master plates were allowed to develop at 30° C for 24 hours and then the colony imprints were made on velveteen which was securely held on a wooden cylinder which had a diameter slightly smaller than the size of a Petri dish bottom. The imprints were then replicated onto plates containing various types of selective or differential media. These plates were incubated at 30° C for 24 hours and the degree of growth and other characteristics such as pigmentation, colony morphology and general appearance were recorded.

All microorganisms were classified according to their growth responses on Bacto-EMB Agar, Bacto-SS Agar and Bacto-Staphylococcus Medium No. 110 (S110). The growth characteristics on these plates, determined by already characterized stock cultures, are given in Table I. Bacto-Potato Dextrose Agar of pH 3.5 was also used to select yeast colonies. A non-selective media was included as the last plate in order to verify the successful transfer onto all the preceding plates.

TABLE I. MICROBIAL FLORA CLASSIFICATION

Group	Growth			Genera included
	EMB	SS	S110	
A	+	+	-	Pseudomonas, Escherichia, Aerobacter, Proteus, Vibrio
B	±	-	+	Staphylococcus, Sarcina, Achromobacter, Flavobacterium
C	+	+	+	Aeromonas
D	-	-	-	Bacillus, Micrococcus
E	+	-	-	Some Pseudomonas and Flavobacterium

Combined Treatments

Antimicrobial Agents

The following antimicrobial agents were examined: Sodium benzoate (Baker USP), potassium sorbate (Sentry brand by Union Carbide), and the sodium salts of the methyl and propyl esters of para-hydroxybenzoic acids (MPB and PPB, by Washine Chemical Company).

Effects of Antimicrobial Agents

TN agars containing concentrations ranging from 0.05 to 10.0% of sodium benzoate, potassium sorbate, MPB and PPB were prepared. Master plates were prepared as usual by picking reference cultures of 40 microbial species from 15 genera onto TN agar. These were

then replicated onto agars containing serial concentrations of antimicrobial agents. The minimum concentrations of chemicals which inhibited the given species of microorganisms were determined by examining growth after incubation at 30° C for 24 hours.

Combined Effect

In order to determine the nature of the combined effect, the samples were prepared as follows:

1. A control sample which was not irradiated and to which no antimicrobial agent was added.
2. A non-irradiated sample to which the antimicrobial agent was added (antimicrobial agent control).
3. An irradiated sample to which no agent was added, (irradiation control).
4. An irradiated sample to which the agent had been added before irradiation.
5. An irradiated sample to which the agent was added after irradiation.

The samples which contained antimicrobial agents were prepared by adding a 10% stock solution to the ground fish to yield a final concentration of 0.1%.

RESULTS AND DISCUSSION

Microbial Spoilage of Dover Sole

Rate of Microbial Spoilage

Ground samples of dover sole (Microstomas pacificus) spoiled rapidly. The longest time interval that any sample required to reach a microbial count of 10^6 microorganisms per gram of flesh, which was arbitrarily chosen as the spoilage point, was two days at a storage temperature of 43° F.

The length of time for spoilage to take place seems to be dependent upon the initial microbial load. The sample with the highest initial microbial load, 3.9×10^5 microorganisms per gram of fish flesh took less than one day to reach a count of 10^6 microorganisms per gram. The sample with the least number of microorganisms per gram required two days (Table II).

Microbial Flora Shift During Storage

The majority of the microorganisms found in fresh dover sole were Gram negative rods of the genera Pseudomonas and Flavobacterium. Some of the metabolically diverse species of the above genera belong to group A. These microorganisms were able to grow on EMB and SS agars and the growth in general was rapid at refrigeration temperatures. The slower species which grow on EMB but

TABLE II. SPOILAGE OF GROUND DOVER SOLE FILLETS AT 43° F

Initial microbial count (microorganisms/gram) x 10^4	Number of days storage to reach 10^6 microorganisms/gram
2.0	2
8.1	1
3.7	1
6.2	1
39.0	<1

not on SS were classed under group E. These two groups dominated the nonirradiated flora (Table III).

TABLE III. MICROBIAL FLORA OF GROUND DOVER SOLE FILLETS

Days storage at 43° F	No. colonies examined	Percent of flora in each group					
		A	B	C	D	E	Y*
0	31	6	0	0	16	77	0
1	42	17	0	0	10	74	0
3	30	30	0	0	7	63	0

*Y = Yeast

Other workers have obtained similar results from various seafoods. Masurovsky, Voss, and Goldblith (23, p. 231) reported that in haddock fillets over 50% of the microflora consisted of Gram negative organisms belonging to the genera Pseudomonas, Mycoplana, Aeromonas, Achromobacter, Flavobacterium, Vibrio, or various genera within the Enterobacteriaceae. Only about 30% of the initial

commensal or contaminating microflora included such Gram positive organisms as members of the genera Bacillus, Corynebacterium, Microbacterium, Micrococcus, Staphylococcus and other genera within the Micrococcaceae. Shewan and Liston (36, p. 380-381) found that in newly caught North Sea cod and in the fillets cut from the same fish, the flora consisted mainly of Pseudomonas and Achromobacter species. The former genera occupied 50% of the flora and the latter, 40% in newly caught fish. The fillets contained 46% Pseudomonas, 24% Achromobacter, and 20% coryneforms. They also reported that during spoilage of cod at 0° C on ice, the Pseudomonas group gradually increased until 90% of the flora eventually consisted of Pseudomonas species.

Microbial Spoilage of Irradiated Dover Sole

Rate of Microbial Spoilage

The storage life of irradiated dover sole, determined microbiologically, is influenced by the initial microbial load and the radiation level. With a dose of 0.1 Mrad, the shelf life is extended from less than one day to at least five days. In the sample with the same initial microbial load (3.9×10^5 microorganisms per gram) at 0.3 Mrad, the number of days to reach a count of 10^6 was nine, and with 0.5 Mrad it was 14 and 1/2 days. The result of irradiation was not

only a reduction of the initial microbial count, but also a lag period during which no apparent microbial growth occured. The lag phase was followed by a rapid growth phase (Figure I).

The effect of the initial microbial load on irradiated samples can be seen from Table IV. Those samples with lower initial microbial counts had a longer refrigerated storage life than those with higher initial microbial counts. When a sample containing 5.5×10^4 microorganisms per gram was irradiated at 0.5 Mrad, storage life was extended four more days than that obtained with the 3.9×10^5 microorganisms per gram sample.

TABLE IV. MICROBIAL SPOILAGE OF IRRADIATED GROUND DOVER SOLE FILLETS STORED AT 43° F.

Initial load (microorganisms/gram) $\times 10^4$	Days to reach 10^6 microorganisms/gram				
	Mrad				
	0	0.1	0.25	0.3	0.5
2.0	2	7	-	-	-
5.5	1 1/2	6	9	-	18
39.0	<1	5	-	-	14 1/2

Microbial Flora Shift During Storage

The group B microorganisms, which are predominately Achromobacter and Gram positive cocci, became numerous after all levels of irradiation. In contrast to the non-irradiated sample where group A and E organisms increased rapidly during storage, the group B

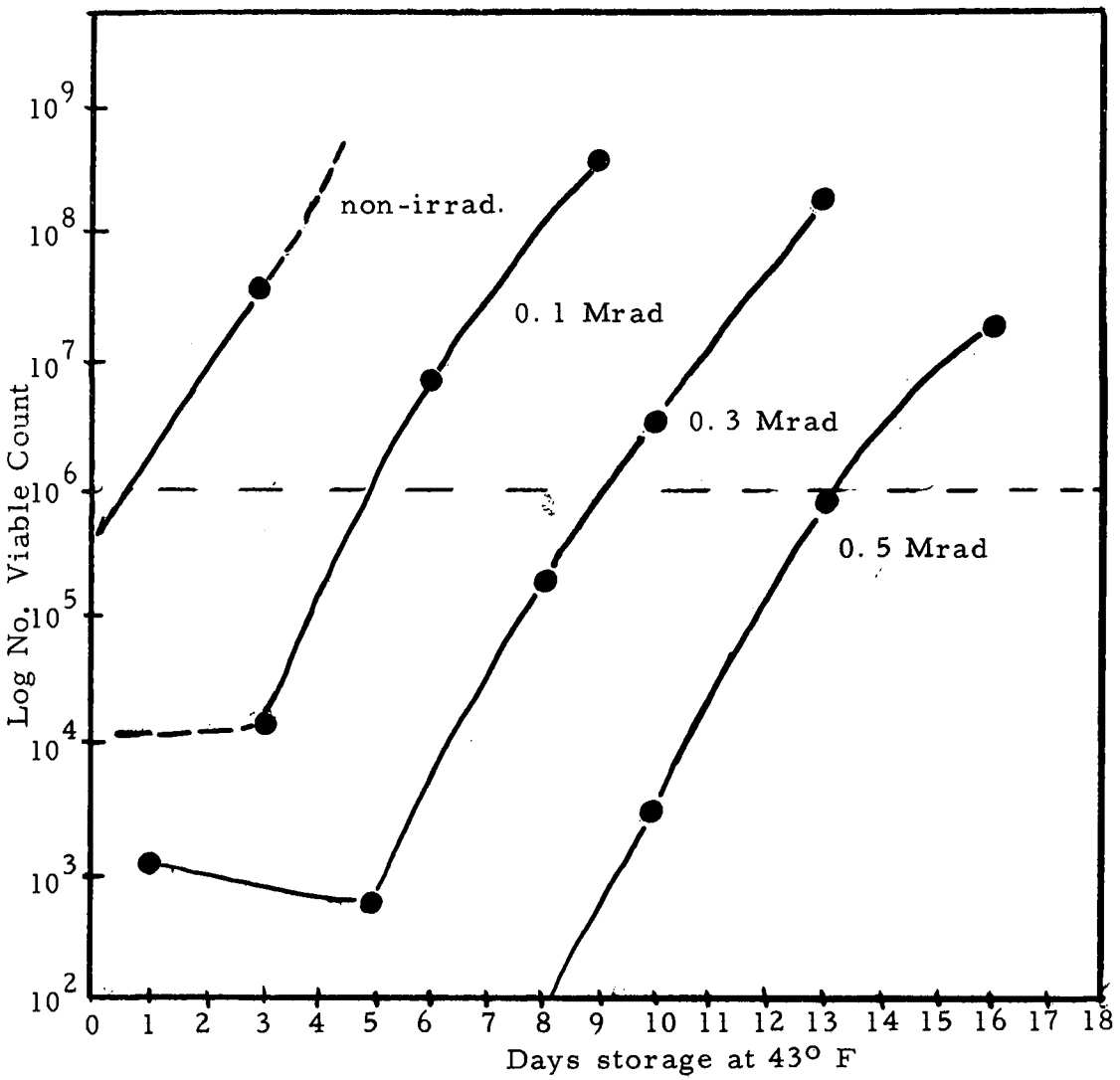

Figure 1. Microbial counts of irradiated dover sole.

organisms which occupied 60-90% of the irradiated flora, did not relinquish the predominant position during storage.

The combination of cold temperatures and radiation created an environment where normally minor groups of microorganisms in the dover sole could contribute to the eventual spoilage. Among them were the Gram positive rods of group D.

It is interesting to compare the data in Table V, which was obtained from fresh samples, with data obtained from samples which had been frozen and thawed before radiation. In Table VI, it can be seen that the initial percentage of yeasts was higher in the frozen samples, but the eventual spoilage was due to the outgrowth of group B organisms after irradiation, as was the case with the fresh sample.

The data obtained in these experiments are in agreement with that presented by Shewan and Liston (36, p. 383) and by Masurovsky, Voss, and Goldblith (23, p. 231). The former authors reported that at radiation doses of 0.25 and 0.5 Mrad, the Pseudomonas types form only about 3 to 5% of the total flora of North Sea cod, while the Achromobacter species predominated. They found, however, that the Pseudomonas would gradually reassert themselves. The latter authors found in irradiated haddock fillets that the great majority of the microorganisms that emerged were members of the Micrococcaceae, spore-forming bacilli, and certain yeasts, molds, and Actinomyces. Corynebacterium, Achromobacter, and Flavobacterium species also

appeared, but in smaller numbers than the bacilli and micrococci. The flora which developed on irradiated marine products during refrigerated storage initially consisted, for the most part, of the more radiation resistant Gram positive microorganisms, but these microorganisms were supplanted, in time, by the more prolific Gram negative psychrophilic organisms that fluorished at the low storage temperature.

TABLE V. MICROBIAL FLORA OF IRRADIATED DOVER SOLE (FRESH).

Dose (Mrad)	Days storage at 43° F	No. colonies examined	Percent flora					
			A	B	C	D	E	Y*
0.10	1	18	0	44	0	11	39	6
	3	15	0	67	0	27	7	0
	6	21	0	76	0	24	0	0
	9	26	0	58	0	12	27	0
0.30	1	20	0	65	0	20	15	0
	8	20	0	95	0	5	0	0
	10	36	0	72	0	3	25	0
0.50	10	12	0	83	0	8	8	0
	16	20	0	75	0	5	20	0

*Y = Yeast

TABLE VI. MICROBIAL FLORA OF IRRADIATED DOVER SOLE (FROZEN AND THAWED)

Dose (Mrad)	Days storage at 43° F	No. colonies examined	Percent flora					
			A	B	C	D	E	Y*
0.30	2	21	0	0	0	0	0	100
	4	20	0	80	0	5	15	0
	6	19	0	89	0	5	5	0
0.35	0	12	0	50	0	16	8	25
	3	19	0	89	0	0	0	11
	6	20	0	90	0	0	0	10
	8	21	0	67	0	19	10	5

* Y = Yeast

Microbial Spoilage of Samples Irradiated in the Presence of Antimicrobial Agents

Rate of Microbial Spoilage

Several food preservatives, when used in combination with radiation, further increased the cold storage life of dover sole. If the aerobic-facultative count of 10^6 microorganisms per gram of fish is denoted as a criteria for the microbial spoilage of fish, the 0.1 Mrad sample required six days storage at 43° F to spoil. When this sample was treated with 0.1% sodium benzoate before irradiation, nine days were required before the same count was reached. The 0.3 Mrad sample required ten days, while the benzoate treated sample lasted 19 days before a 10^6 count was obtained. The sample receiving 0.5 Mrad took 14 days storage to spoil and the benzoate treated sample receiving the same radiation dose lasted longer than 25 days.

As Table VII and Figure 2 show, the prolonged cold storage life of benzoate treated samples upon irradiation was due to the extended dormant periods rather than by reduction of microbial growth rate during the rapid growth phase. When the microbial count started to increase, it did so very rapidly. The rate of microbial propagation was approximately the same in all samples regardless of whether they were irradiated or treated with antimicrobial agents.

A possible mechanism for the antimicrobial action of sodium

TABLE VII. AEROBIC FACULTATIVE COUNTS OF IRRADIATED DOVER SOLE-EFFECT OF SODIUM BENZOATE ADDED BEFORE IRRADIATION.

Days storage at 43° F	Control Mrad				0.1% Sodium benzoate Mrad			
	0	0.1	0.3	0.5	0	0.1	0.3	0.5
0	3.9×10^5	---	---	---	---	---	---	---
1	2.3×10^6	9.4×10^3	8.4×10^2	$<10^2$	1.8×10^6	1.0×10^4	4.4×10^2	$<10^2$
3	6.6×10^7	1.0×10^4	---	---	3.2×10^7	9.4×10^3	---	---
5	---	---	5.0×10^2	$<10^2$	---	---	2.2×10^2	$<10^2$
6	9.0×10^8	6.4×10^6	---	---	2.3×10^9	2.8×10^4	---	---
8	---	---	2.4×10^5	$<10^2$	---	---	2.4×10^2	$<10^2$
9	---	2.8×10^8	---	---	---	1.4×10^6	---	---
10	---	---	3.4×10^6	2.8×10^3	---	---	4.2×10^2	$<10^2$
13	---	---	1.4×10^8	5.5×10^5	---	---	6.0×10^2	$<10^2$
16	---	---	---	2.3×10^7	---	---	2.2×10^5	$<10^2$
20	---	---	---	---	---	---	4.7×10^6	$<10^2$

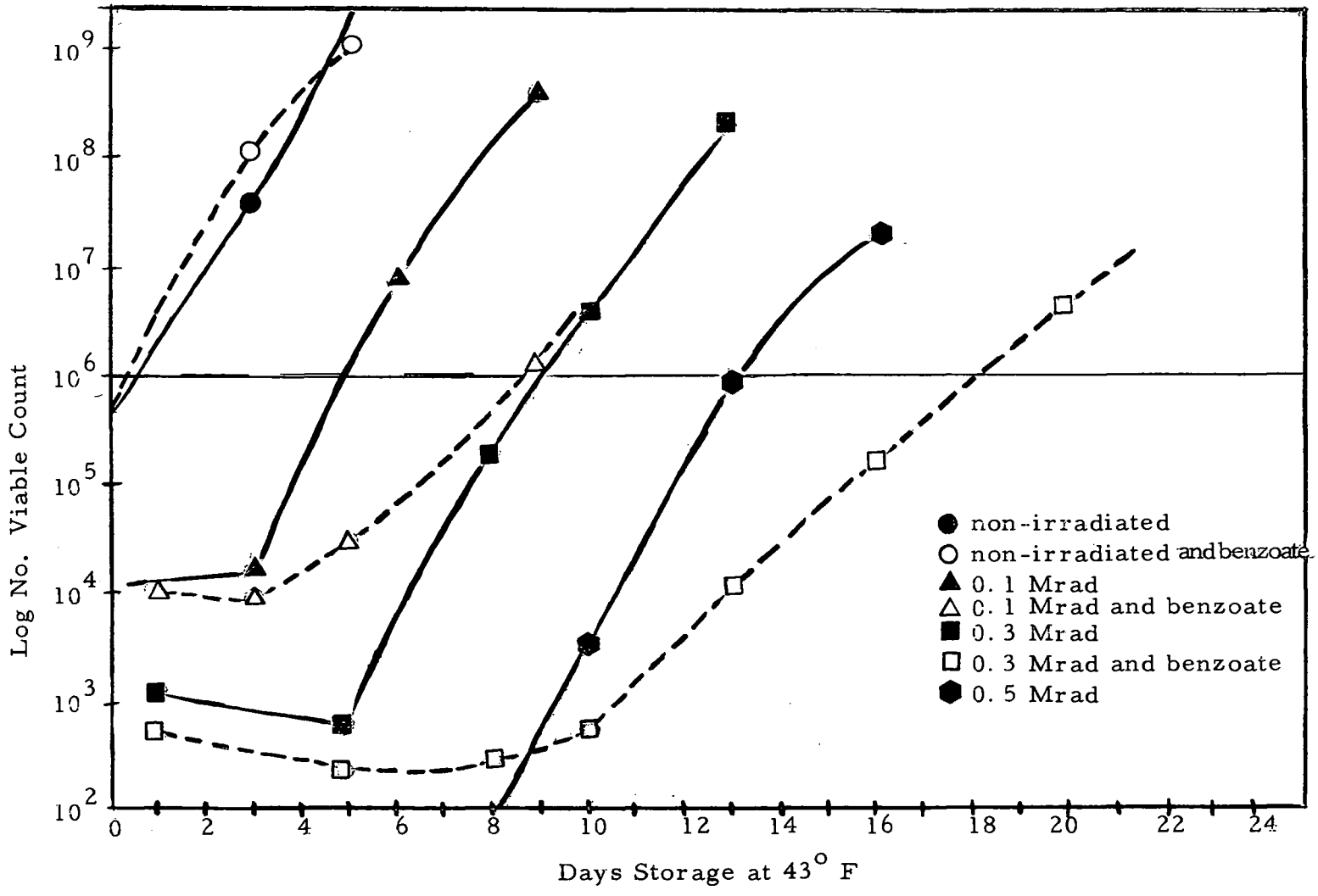

Figure 2. Microbial counts of irradiated dover sole with pre-irradiation addition of 0. 1% sodium benzoate.

benzoate is presented by Bosund (3, p. 544; 4, p. 811-813; 5, p. 1240-1241; 7, p. 819-821). He found that benzoic acid interfered with oxidative phosphorylation in the cell, by a manner similar to that observed with 2, 4-dinitrophenol. The concentrations required to obtain a marked effect were of the same magnitude as those required for a complete suppression of bacterial growth. The presence of benzoic or salicylic acid during glucose and pyruvate oxidation in a Proteus vulgaris culture caused an accumulation of acetate in the medium. The utilization of this compound, as acetyl-S-CoA, or in some other activated form, in synthesis of cell material and as an energy source, was thereby prevented. The important position of "active" acetates in the metabolism of living cells and the fact that benzoic and salicylic acids in the experiments caused the accumulation of acetate at the same concentrations as those required to suppress growth of P. vulgaris suggested that the bacteriostatic effect, at least in part, was due to an interference with the metabolism of the acetyl group. Benzoic acid might have inhibited both respiration and assimilation of metabolites at concentrations affecting growth. Inhibition of terminal respiration might well have been the primary cause of the growth inhibiting effect in a medium containing large amounts of readily utilized substrates such as glucose and amino acids. Benzoic acid was thus claimed to inhibit the biosynthetic processes such as the utilization of acetate for synthesis of metabolic intermediates and the formation

of adaptive enzymes. Bosund, therefore, placed benzoic acid in the same category as other well known "enzyme poisons".

The sodium salts of the methyl and propyl esters of para-hydroxybenzoic acid (MPB and PPB, respectively) had similar effects on the keeping quality of irradiated dover sole (Table VIII and Figures 3 and 4). Using a sample with an initial count of 6.3×10^4 micro-organisms per gram, which took one day to spoil at 43° F, the storage life of the irradiated dover sole without additive was approximately six days. This storage life was extended to 11 days when the samples were irradiated in the presence of MPB and to 16 days in the presence of PPB. In the case of PPB, there were some variations between duplicate samples.

Potassium sorbate also prolonged the storage life of irradiated dover sole. When dover sole was irradiated at 0.35 Mrad in the presence of potassium sorbate, the storage life at 43° F was approximately 16 days. This is in comparison with two days for the non-irradiated sample and six days for the sample which had been irradiated without the use of potassium sorbate. These results are tabulated in Table IX and are shown in Figure 5.

Investigations on the mechanism of the action of sorbic acid by York and Vaughn (48, p. 412-416) resulted in the finding that sorbate interfered with oxidative assimilation, oxidative phosphorylation, and inhibited the sulfhydryl enzymes fumarase, aspartase, and succinic

TABLE VIII. AEROBIC FACULTATIVE COUNTS OF IRRADIATED DOVER SOLE - EFFECTS OF PRE AND POST-IRRADIATION ADDITIONS OF MPB AND PPB.

Days storage at 43° F	Non-Irradiated			Irradiated (0.30 Mrad)				
	No Additive	+ 0.1% MPB	+ 0.1% PPB	No Additive	With MPB	MPB Added	With PPB	PPB Added
0	6.2×10^4	---	---	$<10^2$	$<10^2$	---	$<10^2$	---
1	---	4.6×10^6	4.7×10^6	---	---	$<10^2$	---	(2.6×10^2)*
2	5.4×10^7	---	---	(1.2×10^3)	(1.3×10^2)	---	$<10^2$	---
4	---	---	---	1.6×10^5	---	(7.0×10^2)	---	$<10^2$
5	---	---	---	---	(1.5×10^2)	---	$<10^2$	---
6	---	---	---	6.3×10^6	---	2.8×10^3	---	5.0×10^2 or 8.6×10^3 **
7	---	---	---	---	(4.2×10^2)	---	---	---
9	---	---	---	---	---	1.3×10^5	---	4.7×10^3 or 9.7×10^4
11	---	---	---	---	5.8×10^4	2.7×10^6	(1.7×10^3)	---
13	---	---	---	---	3.2×10^5	---	1.5×10^3	6.6×10^5
15	---	---	---	---	1.6×10^6	---	1.7×10^3 or 3.8×10^5	1.9×10^6 or 1.4×10^7
16	---	---	---	---	---	---	2.8×10^5 or 1.3×10^8	---

*Counts in parentheses were obtained from plates having less than thirty colonies per plate.

**Separate averages were given when the difference between duplicate samples were large.

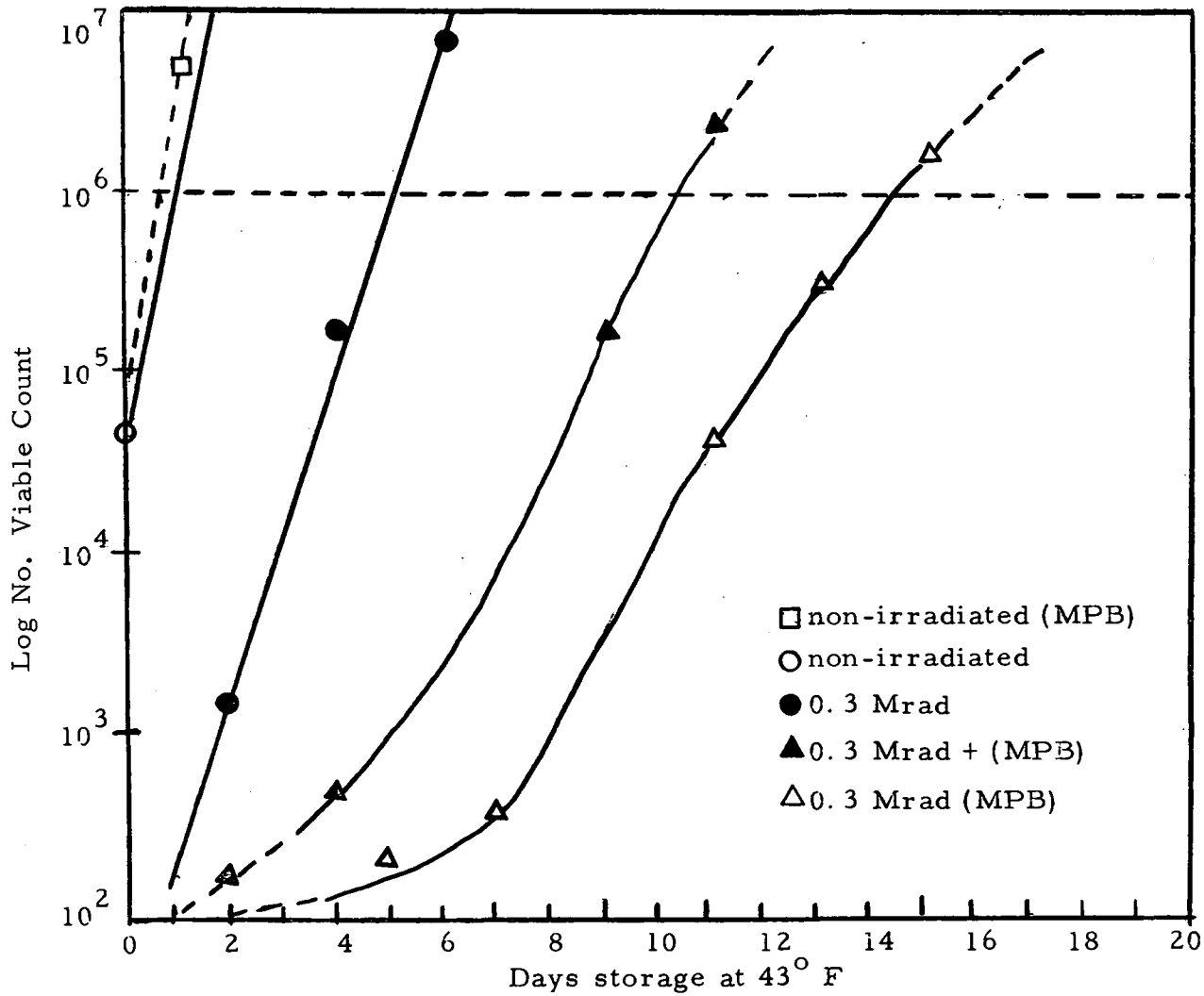

Figure 3. Microbial counts of irradiated (0. 3 Mrad) dover sole with pre- and post-irradiation addition of 0. 1% MPB.

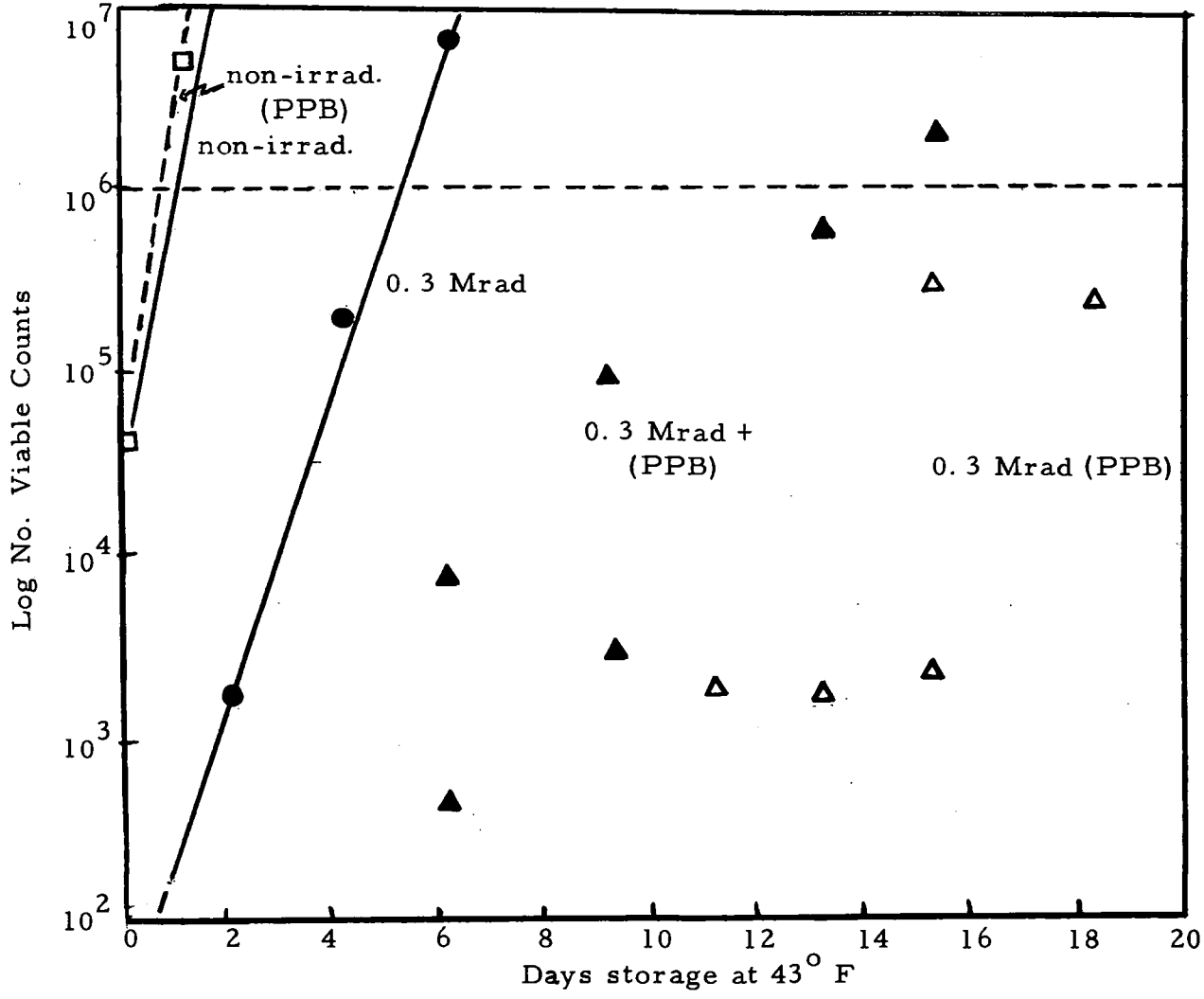

Figure 4. Microbial counts of irradiated (0. 3 Mrad) dover sole with pre- and post-irradiation additions of 0. 1% PPB.

TABLE IX. AEROBIC FACULTATIVE COUNTS OF IRRADIATED DOVER SOLE - EFFECT OF PRE- AND POST-IRRADIATION ADDITIONS OF POTASSIUM SORBATE.

Days storage 43° F	Non-irradiated	Non-Irrad. plus Pot. Sorbate	Irrad. (0.35 Mrad)	Irrad. with 0.1% Pot. Sorbate	Irrad. and 0.1% Pot. Sorbate added
0	3.7×10^4	4.8×10^4	(2.0×10^2)*	(1.7×10^2)	(2.0×10^2)
2	8.0×10^6	1.5×10^7	---	---	---
3	---	---	(3.2×10^2)	(6.0×10^1)	(1.5×10^2)
6	---	---	1.1×10^6	1.5×10^3	6.1×10^3 **
8	---	---	1.2×10^7	6.2×10^4	7.2×10^4
10	---	---	---	5.3×10^5	4.5×10^5
13	---	---	---	7.8×10^5	5.2×10^5
16	---	---	---	3.5×10^6	3.4×10^6 **

*Counts in parentheses were obtained from plates having less than thirty colonies per plate.

**Counts from single samples.

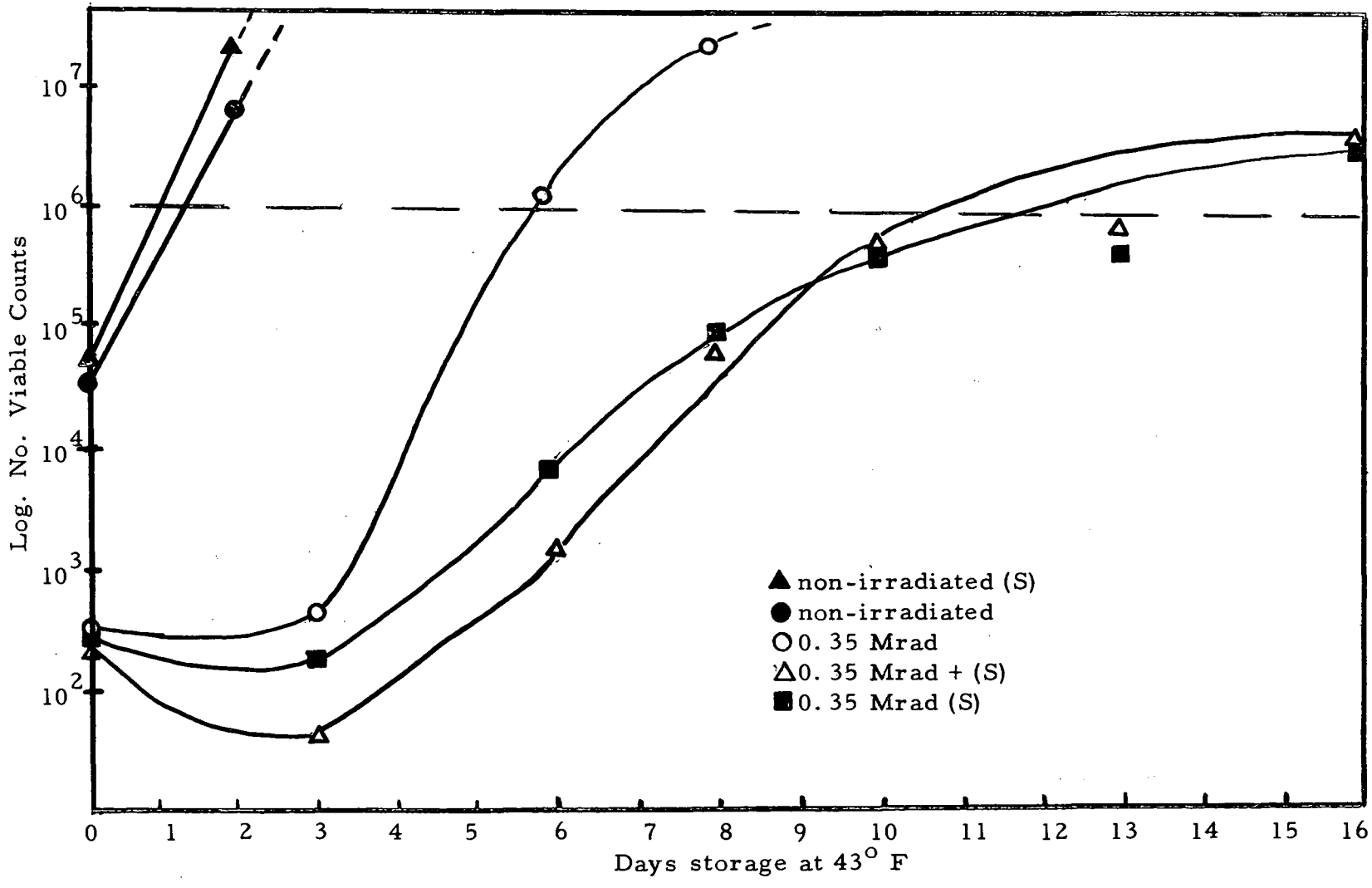

Figure 5. Microbial counts of irradiated (0. 35 Mrad) dover sole with pre- and post-irradiation additions of 0. 1% potassium sorbate.

dehydrogenase.

The numbers of surviving microorganisms as counted immediately after irradiation were not affected by the presence of benzoate. This indicates that benzoate did not act with radiation during the exposure as a "sensitizer". A sensitizing agent was defined by Bridges and Horne (8, p. 105) as an agent which should produce the resulting effect more than the sum of the radiation effect and the agent's effect separately. The combined effect of benzoate and radiation was the same as the effect of radiation alone immediately after irradiation.

A possible explanation for the additive effect is that radiation reduces the microbial population to a fraction of the initial number while the concentration of additive would remain unchanged after irradiation. Therefore, the effective concentration of additive per viable cell would have increased with possible enhancement of antimicrobial effect. Lee, Shiflett and Sinnhuber (17) reported that when the normal flora of dover sole obtained from the flesh fillets were diluted so as to contain 3000, 300, or 30 microorganisms per tube and then incubated at 30° C with or without 0.1% sodium benzoate, there was no increase of bacteriostatic effect of sodium benzoate even when the microbial concentration was drastically reduced. This indicates that the effect of benzoate in prolonging cold storage life of dover sole was not the direct consequence of radiation reducing the initial number of microorganisms.

Another reason for the additive effect could be that the organisms surviving irradiation are, by chance, those which are most sensitive to the above antimicrobial agents. This is shown not to be the case by the data in Table X. The minimum concentration which would produce a microbiocidal effect was determined for the four antimicrobial agents. The microorganisms which are known to be more resistant to radiation were also more resistant to the antimicrobial agents. Staphylococcus aureus could grow at concentrations up to 1.50% benzoate, 5.00% sorbate, and 0.45% MPB: Sarcina lutea and Micrococcus species grew at concentrations up to 5.00%, greater than 10.00%, and 5.00% of benzoate, sorbate and MPB, respectively. These organisms also endured concentrations of PPB up to 1.00% for S. lutea and 0.10% for Micrococcus species. Achromobacter, which represents the majority of radiation survivors in dover sole, was also relatively resistant to the antimicrobial agents. The Pseudomonas cultures which were investigated showed a varied response to the antimicrobial agents. Some were quite resistant and others were very sensitive.

Shewan and Liston (36, p. 383) proposed that radiation appears to have three effects on the bacterial population in fish-- a bactericidal one, a bacteriostatic one and a devitalizing one. There appear to be some bacteria which, after a period of time, recover from the damage caused by radiation and then grow in the normal way. They stated that an additional treatment with antibiotic seemed to prevent

TABLE X. MINIMUM LETHAL CONCENTRATIONS OF SODIUM BENZOATE, POTASSIUM SORBATE, MPB, AND PPB.

Microorganism	Source	Minimum lethal concentration (%)			
		Sodium benzoate	Pot. sorbate	MPB	PPB
Bacillus subtilis	ATCC 9945	0.10	1.20	0.50	<0.05
B. cereus	OSU Mb.	0.50	1.40	0.30	<0.05
B. megaterium	OSU Mb.	1.00	1.10	0.35	<0.05
B. species	U. W. 269[1]	0.80	0.80	0.40	<0.05
Staphylococcus aureus	OSU Mb[2]	1.50	5.00	0.45	<0.05
Sarcina lutea	OSU Mb	5.00	>10.00	5.00	1.00
Micrococcus sp.	U. W. 217	5.00	>10.00	5.00	0.10
Pseudomonas flourescens	OSU Mb	5.00	5.00	0.5	0.4
Ps. putrificiens	OSU Mb	0.10	0.45	1.00	<0.05
Ps. sp.	U.S. 223	0.50	1.50	0.15	<0.05
Ps. (flourescent sp.)	U. W. 218	1.00	1.10	0.30	<0.05
Ps. sp. Type I	NCMB 153[3]	0.90	1.20	0.40	0.25
Ps. sp. Type I	NCMB 406	0.80	1.00	0.30	<0.05
Ps. sp. Type II	NCMB 133	1.00	1.00	0.40	0.20
Ps. sp. Type II	NCMB 320	0.40	0.35	0.20	<0.05
Ps. sp. Type III	NCMB 225	0.50	0.10	0.50	<0.05
Flavobacterium capsulatum	ATCC 14666	0.25	0.6	0.1	<0.05
Ps. sp. Type IV	NCMB 1300	NGTN	0.1	0.05	<0.05
Ps. sp. Type IV	NCMB 334	0.2	0.15	0.1	<0.1
Ps. aeruginosa	OSU Mb	5.0	5.0	0.3	<0.05
Escherichia coli	OSU Mb	5.0	>0.5	0.5	<0.05
Aerobacter aerogenes	OSU Mb	1.0	1.1	0.5	0.1
Achromobacter sp.	NCMB 131	0.9	0.25	0.1	<0.05
Achromo. sp.	NCMB 132	1.5	0.4	0.1	<0.05
Aeromonas formicans	NCMB 23	0.5	0.4	0.5	<0.05
Aerom. hydrophila	NCMB 72	0.45	0.2	0.5	<0.05
Vibrio sp.	U. W. 371	0.5	0.1	0.5	0.1
Burgundy yeast	OSU Mb	1.1	0.5	0.1	<0.05
Champagne yeast	OSU Mb	1.5	>0.5	0.1	<0.05

[1]University of Washington, College of Fisheries Collection. Seattle, Washington. Kindly provided by Dr. J. Liston.
[2]Stock cultures of Dept. of Microbiology, Oregon State Univ. Corvallis, Oregon.
[3]National Collection of Marine Bacteria, Torry Research Station. Aberdeen, Scotland; kindly provided by Dr. J. M. Shewan.

this recovery process. A similar mechanism may have been involved for the sodium benzoate and other antimicrobial agents discussed.

According to Bosund (6, p. 794-799), benzoic acid has little microbiocidal activity at a neutral pH. He showed that the ineffectiveness of benzoate was due to the inability of the cell to incorporate this compound at this pH, by demonstrating little or no incorporation of benzoic acid into P. vulgaris and Baker's yeast near a pH of 7.0. The pH of the fresh fillets was 6.8 and this did not change either by benzoate treatment or irradiation. Since radiation did not change the pH of dover sole, it may be possible that the irradiation had altered the cellular permeability so that benzoate could be passed into the cell despite the neutrality of the pH.

Reports of permeability changes in cells caused by irradiation have been made by Srb (39, p. 309-311) and Tskukamura (46, p. 113). Srb, using the inner epidermis of the third coat of onion seedlings, studied the rate of plasmolysis as a response to radiation exposure. He found that X-irradiation seriously damaged cell permeability. Tsukamura, using Mycobacterium and ultraviolet radiation (u. v.), found that the u. v. irradiated cells showed an increased incorporation of P-32 phosphate and S-35 sulfate. The possibility of membrane damage was suggested because the cells lost their acid-fastness after u. v. irradiation. The possibility that damage to membranes contributed to the killing of lymphocytes by radiation was indicated by

electron microscope studies by Alexander and Bacq. (1, p. 17). They proposed that a mechanism by which oxygen enhanced the radiation effect could be envisaged for membrane damage. The unsaturated fat-containing phospholipid membrane may undergo a chain reaction with oxygen which is initiated by an ionization.

Microbial Flora Shift During Storage

At the lower dose rate (0.1 Mrad), sodium benzoate did not appreciably change the type of flora causing spoilage. Spoilage was mostly due to group B and E organisms, although there were slightly more group D organisms present than in the untreated irradiated sample. At the higher radiation levels (0.3 and 0.5 Mrad) the spoilage in samples to which sodium benzoate had been added was due mainly to yeasts. The microbial flora in the 0.5 Mrad sample with benzoate were all yeasts.

Potassium sorbate did not eliminate the yeast spoilage as was expected. At a radiation level of 0.35 Mrad, the spoilage in the presence of potassium sorbate was due exclusively to yeasts. The MPB and the PPB, on the other hand, appeared to be somewhat more effective against yeasts. Although the yeast count was high in the early days of storage, they did not dominate the final spoilage flora of MPB and PPB treated samples. In the case of these two agents the spoilage was still caused by the group B microorganisms.

Microbial Spoilage of Irradiated Samples Post-Treated with Antimicrobial Agents

Rate of Microbial Spoilage

More evidence that these agents did not act as sensitizers was obtained when sodium benzoate and potassium sorbate, added immediately after irradiation, had the same effect as when the agents were added before irradiation. Table XI and Figure 6 show that the sample which was irradiated in the presence of benzoate and the sample to which benzoate was added after irradiation reached a count of 10^6 microorganisms per gram in about 14-15 days. Similar results were obtained with potassium sorbate, (Table IX). With a sample that reached a count of 10^6 microorganisms per gram in six days after irradiation alone, both types of treatment with potassium sorbate resulted in spoilage within 16 days.

The reaction of MPB and PPB was slightly different. These agents were somewhat more effective when added before irradiation than when added after. In a sample which took approximately six days to spoil after irradiation, 15 days were required for spoilage when MPB was added before irradiation, compared with 11 days for the sample which was irradiated before the addition of MPB. When PPB was added before radiation, approximately 16 days elapsed before the count reached 10^6 microorganisms per gram, while 15 days were

TABLE XI. AEROBIC FACULTATIVE COUNTS OF DOVER SOLE - EFFECT OF PRE- AND POST-IRRADIATION ADDITIONS OF SODIUM BENZOATE.

Days storage at 43° F	Aerobic-facultative count/gr				
	Non-Irradiated		Irradiated (0.30 MR)		
	No Benz.	Benz.	No Benz.	In Benz.	Benz. added
0	3.9×10^4	---	---	---	---
1	8.1×10^4	6.2×10^4	$\sim$ 100	>100	350
4	4.8×10^8	3.6×10^8	1.3×10^3	3.2×10^2	>100
6			1.6×10^5	---	---
7				2.7×10^3	5.3×10^2
10				1.5×10^4	5.7×10^3
12				3.3×10^5	1.2×10^5
14				1.9×10^6	6.0×10^5

required for the sample to which PPB was added after irradiation. These results are illustrated by Figures 2 and 3.

Microbial Flora Shift During Storage

The microbial flora of the samples to which potassium sorbate was added after irradiation was dominated by yeasts after storage. The same was true for the samples which had been irradiated in the presence of sorbate. The spoilage of the MPB and the PPB treated samples, which were added both before and after irradiation, was due mainly to group B microorganisms. These results are presented in Tables XII and XIII.

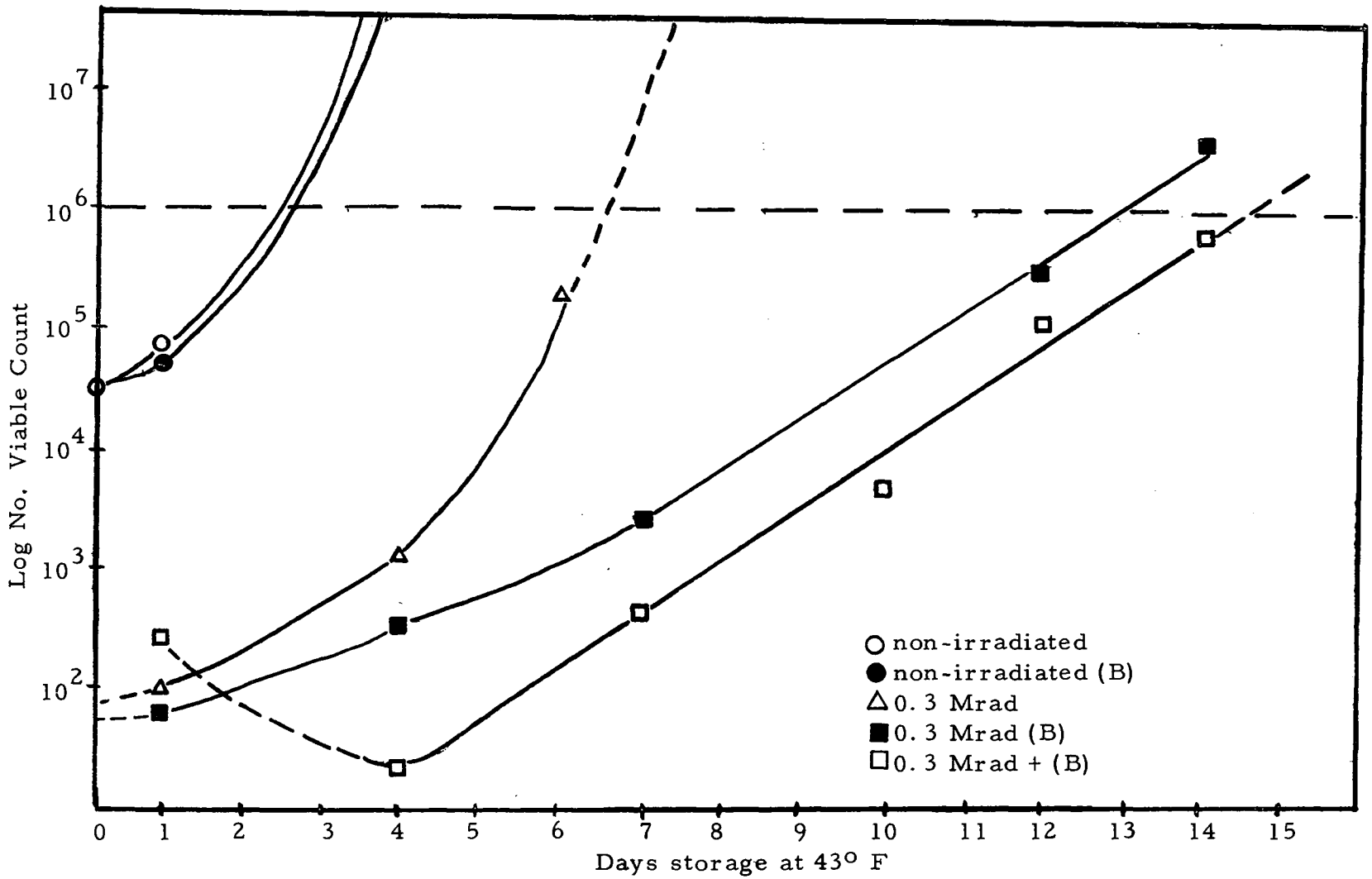

Figure 6. Microbial counts of irradiated (0. 3 Mrad) dover sole with pre- and post-irradiation additions of 0. 1% sodium benzoate.

TABLE XII. MICROBIAL FLORA OF DOVER SOLE IRRADIATED WITH POTASSIUM SORBATE.

Treatment / Flora / Storage days	Non-Irrad. plus Pot. Sorbate			Irrad. (0.35 Mrad)			Irrad. With 0.1% Pot. Sorbate			Irrad. and 0.1% Pot. Sorbate Added		
	No. Colonies Examined	% Yeasts	% Bact.	No. Colonies Examined	% Yeasts	% Bact.	No. Colonies Examined	% Yeasts	% Bact.	No. Colonies Examined	% Yeasts	% Bact.
0	15	6	94	12	25	75	8	13	87	--	--	--
2	20	0	100	--	--	--	--	--	--	--	--	--
3	--	-	--	19	11	89	--	--	--	8	38	62
6	--	-	--	20	0	100	15	100	0	21	38	62
8	--	-	--	21	5	95	19	100	0	20	100	0
10	--	-	--	--	--	--	20	100	0	21	90	10
13	--	-	--	--	--	--	21	100	0	21	100	0
16	--	-	--	--	--	--	21	100	0	21	100	0

TABLE XIII. MICROBIAL FLORA OF DOVER SOLE IRRADIATED WITH MPB AND PPB.

	System	Additives (0.1%)	Days storage (43°F)	No. of Colonies Examined	% Microbial Flora[1] A	B	C	D	E	Yeasts
A.	Non-irradiated	0	2	21	90	0	0	0	0	10
	controls	MPB	1	21	81	0	5	0	14	0
		PPB	1	21	90	0	0	10	0	0
B.	Irradiated	0	2	20	0	0	0	0	0	100
	(0.3 Mrad)	0	4	20	0	80	0	5	15	0
	Control	0	6	19	0	89	0	5	5	0
C.	MPB or PPB	MPB	7	21	0	14	0	10	14	62
	Present during	"	11	19	5	5	0	0	0	89
	Irradiation	"	13	21	0	71	0	14	0	14
		"	15	18	0	81	0	0	0	17
		PPB	7	21	0	19	0	10	61	10
		"	11	16	0	25	0	19	0	45
		"	13	15	0	0	0	0	0	100
		"	15	16	0	19	0	0	0	81
		"	18	19	0	79	0	0	10	10
D.	MPB or PPB	MPB	4	20	0	65	0	15	0	20
	Added to	"	6	19	0	58	0	5	0	37
	Irradiated	"	9	21	0	100	0	0	0	0
	Samples	"	11	14	0	86	0	0	14	0
		PPB	1	7	0	57	0	0	0	43
		"	4	7	0	43	0	0	57	0
		"	6	8	0	38	0	50	13	0
		"	9	16	0	100	0	0	0	0
		"	11	21	0	0	0	0	100	0
		"	13	21	0	76	0	0	0	24
		"	15	18	0	94	0	0	0	0

[1]Majority of group A bacteria belong to genus Pseudomonas and group B to Achromobacter.

Microbial Spoilage of Chemically Treated Samples

Rate of Microbial Spoilage

None of the additives described above had any effect on the storage life of dover sole when used without radiation. Upon storage at 43° F, the microbial count increased as rapidly as the control samples. The characteristic putrid odor of spoiled fish, however, was reduced in the benzoate treated samples. Stewart and Castell (38, p. 595-596) found that 0. 1% sodium benzoate and benzoic acid inhibited the trimethylamine production by some marine bacteria. This inhibition occurred in neutral pH where benzoic acid and sodium benzoate were almost completely dissociated and not expected to have any antimicrobial effect.

Tarr (43, p. 204-205) stated that the reaction of fish flesh is normally not sufficiently acidic for benzoic acid to exert any significant antimicrobial activity. In spite of this fact, sodium benzoate is at present, a common agent commercially used as a dip for fishery products. Fellers and Harvey (11, p. 12) reported in 1940 that a benzoated brine has a cleansing and firming effect on fish fillets.

Sorbic acid is used mainly as a fungistat for the control of yeasts and molds in various products. Niven and Chesbro (26, p. 859) found that although sorbic acid apparently had no effect on the spoilage rate of fresh beef when used alone, it extended the storage life when used

with radiation and with radiation and antibiotics.

Microbial Flora Shift During Storage

The eventual spoilage was due mainly to the outgrowth of group A microorganisms, despite the presence of any of the four agents tested. This is the same group responsible for the spoilage of the untreated dover sole.

Comparative Effectiveness of the Antimicrobial Agents

Since the chemical agents tested did not act as radiation sensitizers, the dose modification factor (DMF) commonly used for comparing the effectiveness of sensitizing agents cannot be used. It would be appropriate, therefore, to introduce a term to compare the effectiveness of the agents. This term is the "storage extension factor" (SEF) first proposed by Lee (17) at the Fourth Annual Contractors Meeting on AEC Radiation Pasteurization of Foods. The SEF is defined as the days of cold storage obtainable with a combined process divided by the days of cold storage obtainable with the same dose of radiation alone. The SEF values for all four agents were approximately two, or the irradiated shelf life was doubled. The SEF appears to be dependent on the radiation dose. The SEF increased slightly when higher radiation doses were employed. The SEF values for the four antimicrobial agents are presented in Table XIV.

TABLE XIV. STORAGE EXTENSION FACTOR (SEF) VALUES FOR VARIOUS AGENTS TESTED.

Preservative (0.1%)	Radiation (Mrad)	SEF
Sodium benzoate	None	1.0*
Sodium benzoate	0.1	1.8
Sodium benzoate	0.3	2.0
Sodium benzoate	0.5	2.1
Potassium sorbate	None	1.0
Potassium sorbate	0.35	2.0
MPB	None	1.0
MPB	0.3	1.8 - 2.5**
PPB	None	1.0
PPB	0.3	2.0 - 2.1**

*SEF of 1.0 indicates no storage extension.

**Increased SEF was noted when added before irradiation.

SUMMARY AND CONCLUSIONS

The combined effect of ionizing radiation and four antimicrobial agents on extending the 43° F storage life of dover sole was determined. The results and conclusions may be summarized as follows:

1. Sodium benzoate, potassium sorbate, the sodium salts of the methyl and propyl esters of para-hydroxybenzoic acid (MPB and PPB) approximately doubled the storage life at 43° F of irradiated dover sole. This effect was due to an extension of the dormant phase of microbial growth which follows radiation exposure rather than to a reduction of the growth rate in general.
2. The effect of these agents were the same whether added before or after irradiation. This, and the fact that the number of microorganisms surviving irradiation was unchanged by the presence of the agents, indicates that the antimicrobial agents did not act as "sensitizers".
3. The preservatives had no effect on the storage life of unirradiated dover sole. Tests with reference cultures showed that most microorganisms were not sensitive to 0.1% of sodium benzoate, potassium sorbate, MPB or PPB.
4. The agents did not change the microbial spoilage pattern at any radiation level except 0.5 Mrad. In this sample, growth

of yeasts was noted.

5. The antimicrobial agents appear that they may prevent the recovery of radiation injured microorganisms which would have ordinarily recovered from such damage in food during storage.

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