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PASTEURIZATION OF FISHERY PRODUCTS WITH GAMMA RAYS FROM A COBALT-60 SOURCE

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ABSTRACT

This paper describes research on radiation-pasteurization of fresh fish so as to extend its shelf life. The U.S. Bureau of Commercial Fisheries Technological Laboratory in Gloucester, Mass., conducted the research under a contract from the U.S. Atomic Energy Commission.

The results indicate that a number of economically important North Atlantic fishery products can be held refrigerated in an acceptable condition for at least one month after treatment with low doses of gamma radiation without significant nutritive losses. The method is practical even when 50 percent of the shelf life has been used up.

An investigation of the chemistry of fish flavors and odors has not, thus far, uncovered any evidence that irradiation causes the formation of aberrant or unusual compounds.

Packaging studies showed that many commercially available plastic materials are suitable for packaging radiopasteurized fishery products and that in most cases unsuitability was apparently due to high gas permeability rates and poor sealing characteristics.

Total plate counts conducted before and after irradiation with 250,000 rads indicated that the bacterial flora in haddock fillets was reduced by approximately 99 percent.

SUMMARY

Research on radiation-pasteurization of fresh fish to extend its refrigerated shelf life conducted by the U. S. Bureau of Commercial Fisheries Technological Laboratory at ester, Mass., under a contract from the U. S. Atomic Energy Commission.

The results thus far indicate that clam meats and haddock, cod, pollock, and ocean perch ts can be held refrigerated in an acceptable condition for at least one month after treat- t with low doses of gamma radiation (150-450 kilorads).

The effect of irradiation on amino acids and B-vitamins was relatively insignificant and inly not greater than the effects of cooking or of seasonal variations.

Suitable techniques for studying volatile carbonyl and sulfide compounds in clam meats fish fillets have been developed and changes in the concentrations of carbonyls and sul- s, caused by irradiation, storage, and cooking, have been determined. At least 20 aldehydes, nes, and sulfides have been identified in the volatiles of clam meats.

Data have been obtained to show that radiation-pasteurization of haddock fillets has prac- l application even when the fillets have been stored in ice for more than half their normal f life prior to irradiation.

Experiments to determine the suitability of available plastic films or wrappings as pack- g materials for irradiated fishery products showed that films having a relatively low oxy-

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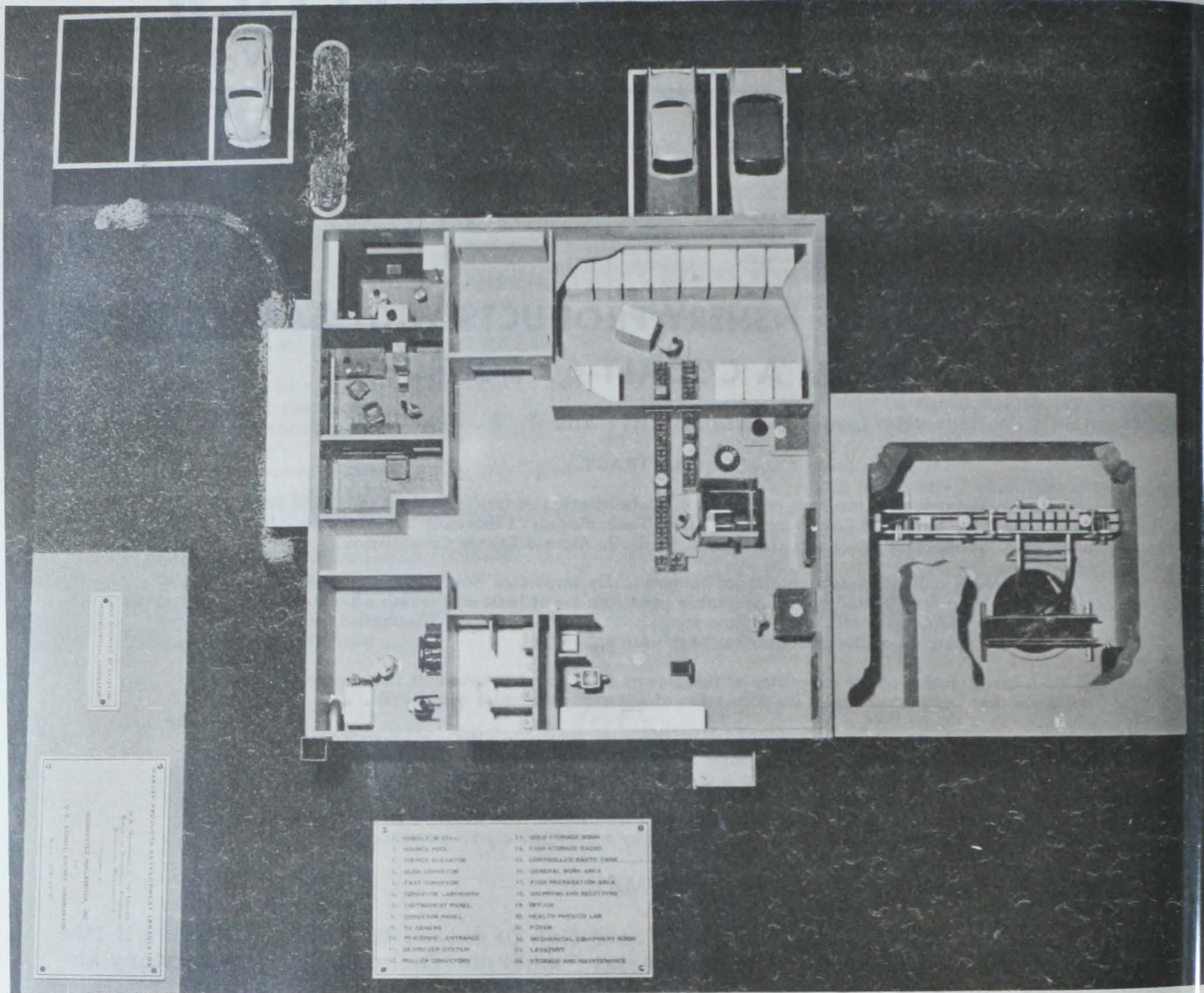

Fig. 1 - Model of Marine Products Development Irradiator.

gen permeability are suitable and those having a relatively high oxygen permeability are un-suitable. The films tested (polyethlene, polypropylene, polyester, nylon-11, and others) we[re] found to be resistant to bacterial penetration. They were also relatively free from pinholes and their seams were adequately strong.

It was found that the bacterial numbers in fresh haddock fillets were reduced by at leas[t] 99 percent by irradiation with 250,000 rads.

BACKGROUND

The value of fishery products as a source of protein is well known and since man's dependence on them is anticipated to increase, it is inevitable that he apply his latest technology to the sea. In general, fishery products are relatively perishable and consequently distribution of fresh fish and shellfish is limited to coastal areas. Wider distribution is possible when those products are either heat-processed or frozen; however, in most cases, fresh fish and shellfish, like fresh fruits and vegetables, command a higher consumer preference and a higher selling price than their frozen counterparts.

Since the discovery that ionizing radiations can be used to preserve food, much work ha[s] been done, especially in recent years, on the use of this energy for sterilization of many foo[ds] including fish and shellfish. The application of high levels of energy to those products resulted in significant quality loss from irradiation-induced flavors and odors. However, earl[y] earch indicated that the refrigerated storage life of fishery products could be significant- extended without objectionable quality changes when irradiated with pasteurizing doses of zing radiations (less than one megarad).

This paper reports on the results of research at the Bureau of Commercial Fisheries nological Laboratory in Gloucester, Mass., on pasteurization of fishery products with na rays from cobalt-60. Objectives were to determine:

(1) The commercial species of Atlantic fish and shellfish to which radiation-pasteurization is suitable.

(2) The optimum level of radiation for each species. The optimum level might be defined as that dose which permits a significant extension in the refrigerated shelf life of the product without causing objectionable alterations in product quality. A marketing survey conducted in 1960 indicated that if fish and shellfish could retain their fresh quality for 3 to 4 weeks, their distribution could be extended to all points in the United States. Hopefully, this would result in increased per capita consumption of fishery products, especially fresh fish.

(3) The effect on quality of irradiating the products in aerobic (oxygen) and anaerobic (non-oxygen) environments.

(4) The effect on shelf life of storage at near-freezing temperature (32^{o}-35^{o} F.) and at the temperature of domestic refrigerators (about 42^{o} F.).

(5) The effects of irradiation, cooking, and extended storage on the concentration of free and total amino acids and B-vitamins.

(6) Changes in composition of compounds as a result of irradiation and extended storage.

(7) The suitability of available flexible packaging materials as containers for irradiated fishery products.

(8) The effect of pre-irradiation quality of fish on post-irradiation shelf life.

THE RESEARCH PROGRAM

OPTIMUM DOSE AND ACCEPTANCE STUDIES: The work schedule to determine the ess feasibility and optimum dose levels was designed to permit investigations at the rate o species a year. Haddock fillets (*Melanogramus aeglefinus*) and soft-shell clam meats *arenaria*) were studied first.

Experiments were conducted to determine:

(1) The optimum dose for air- and vacuum-packed products.

(2) Their storage life at 33^{o} F. and at 42^{o} F.

(3) Their level of acceptability during storage after irradiation at the optimum dose.

To determine optimum doses, the samples were packed in cans in air or under vacuum, diated at different doses and stored at 33^{o} F. and at 42^{o} F. Periodically the organoleptic ity of the samples was compared with that of fresh or fresh frozen samples. At the end ach experiment, the average organoleptic score of the samples from each of several dose ls was compared with the average score of the control samples. That dose that permit- the product to be held for 30 days and that resulted in scores least different from the control samples was considered to be the optimum dose. Student's t-test was applied to determine the degree to which the irradiated products differed from the controls.

To determine acceptability, samples were air-packed (when the optimum dose studies did not indicate a necessity for vacuum-packing), irradiated at the optimum dose, stored at 33° F., and periodically examined organoleptically by the single stimulus method using a nine-point hedonic scale.

Table 1 - Optimum Radiation Levels for Some Fishery Products

Product	Air-Packed in Cans	Vaccum-Packed in Cans
	Rads	Rads
Haddock fillets	250,000	150,000
Clam meats	450,000	350,000
Pollock fillets	150,000	150,000
Ocean perch fillets . .	250,000	150,000
Cod fillets.	150,000	-

Details of the procedures used have been published by Connors et al (1964).

Species investigated to date are haddock, soft-shell clams, pollock (Pollachius virens), ocean perch (Sebastes marinus), and cod (Gadus morhua). The optimum dose data for those species are shown in table 1.

To determine acceptability, haddock fillets and clam meats were taste-tested by trained and untrained sensory panels. The results are shown in tables 2 and 3.

Table 2 - Average Organoleptic Scores Assigned to Irradiated Deep-Fat-Fired Haddock Fillets by Different Taste Panels

Panel	No. in Panel	Storage Time at 33° F. Prior to Testing	Average1/ Organoleptic Score of Treated Samples	Average1/ Organoleptic Score of Control Samples
		Days		
Gloucester	12	15	8.0	2/
Mixed	45	15	8.4	2/
U.S. Army	19	60	7.5	8.0
U.S. Army	300	15	5.5	6.5
U.S. Army	300	30	5.6	6.1

1/A 9-point hedonic scale was used where: 9 - like extremely, 8 - like very much, 7 - like moderately, 6 - like slightly, 5 - neither like nor dislike, 4 - dislike slightly, 3 - dislike moderately, 2 - dislike very much, 1 - dislike extremely.
2/No controls were used in these tests, but previous experience indicates that controls would have received scores between 8 and 9.

The results to date show that radiation with cobalt-60 gamma rays significantly extends the refrigerated storage life of haddock, pollock, ocean perch, and cod fillets and clam meats without creating objectionable changes in the organoleptic quality of the product.

The data in table 1 indicate that air-packed samples generally required a higher irradiation dose than did the vacuum-packed samples. This is probably explainable on the basis that the organism mainly responsible for spoilage requires an aerobic environment.

Table 3 - Average Organoleptic Scores Assigned to Irradiated Deep-Fat-Fried Soft-Shell Clam Meats by Different Taste Panels

Panel	No. in Panel	Storage Time at 33° F. Prior to Testing	Average1/ Organoleptic Score of Treated Samples
		Days	
Gloucester	12	15	7.8
Gloucester	12	30	7.7
Mixed	45	15	8.6
M.I.T.	?	15	6.0
M.I.T.	?	30	5.1

1/A 9-point hedonic scale was used where: 9 - like extremely, 8 - like very much, 7 - like moderately, 6 - like slightly, 5 - neither like nor dislike, 4 - dislike slightly, 3 - dislike moderately, 2 - dislike very much, 1 - dislike extremely.

The organoleptic test results show that the taste panel did not detect statistically significant quality differences between air-packed and vacuum-packed samples. However, comments by individuals gave evidence that air-packed clam meats were more tender than the vacuum-packed samples. With ocean perch fillets there were comments of rancidity in some air-packed samples, but this was not severe enough to be objectionable.

AMINO ACID AND B-VITAMIN STUDIES: Haddock fillets and soft-shell clam meats were analyzed for total and free amino acids and for B-vitamins to determine the effects of radiation, cooking, and storage on those nutrients.

Analyses were made on unirradiated fresh samples, samples irradiated at their optimum dose level, samples irradiated at ten times their optimum dose level, stored samples, heat-processed samples, and samples which received a combination of treatments. The procedure used for the quantitative analyses and the results are reported by Brooke et al (1964).

Irradiation at the optimum dose levels did not significantly affect the amino acid and vi-ain-B values of haddock fillets and clam meats. There was some loss of thiamine. But en when the samples were irradiated at a level ten times greater than the optimum dose, nutrients were not affected to any greater degree than can be expected as a result of cook-or seasonal variations.

FLAVOR AND ODOR STUDIES: Because fishery products spoil relatively quickly and use quality deterioration is detectable in volatile components (as evidenced by bad odors flavors), a project was started to study the volatile compounds in fish and shellfish.

Samples were diced and the volatile compounds were removed under high vacuum and ensed in a liquid nitrogen-cooled trap. The condensate was then heated and the vapors made to flow into a transfer chamber of measured volume which was then connected to s chromatograph to permit analysis of the volatiles. Two gas chromatographs were a-able for this work. The instrument used for the early work was fitted with a Strontium-90 zation detector. The retention column is fitted with a temperature programmer. The mn temperature can be automatically increased from the temperature of dry ice (~-79° C.) bout 70° C., at a predetermined rate. The other gas chromatograph is equipped with a hy-gen flame detector and the retention column temperature can be automatically programmed m room temperature to 500° C.

Several retention columns were used, all with the same solid support--acid washed omosorb-W. The liquid phase or column coatings used were:

(1) 2, 5, or 10% oxydipropionitrile.

(2) 15 or 25% Carbowax 20M.

(3) 5% butanediol succinate.

(4) 20% ethylene glycol succinate.

Chromatograms resulting from the analyses were studied to note which compounds re-ted effects of irradiation, heat-processing, storage, or a combination of treatments (see re 1 for a typical chromatogram).

In addition to studying total volatiles, classes of compounds were studied separately. Car-l compounds were analyzed by two methods:

(1) A modification of the Girard-T reagent method, described by Gaddis et al (1964) which permitted gas chromatography of isolated carbonyl compounds, was used to separate and identify individual carbonyls.

(2) Total carbonly compounds were determined by the 2,4-dinitrophenylhydrazine precipitation method described by Mendelsohn and Steinberg (1962).

Although more work needs to be done, enough evidence has been obtained to show that the atile carbonly compounds in clam meats may be useful indicators of quality and/or treat-nt of the product. Figure 1 represents a typical gas chromatogram of the concentrated atile carbonyls in fresh raw clam meats. It is believed that this type of chromatogram be used as a standard for identifying fresh clams, but more data are required before this be established. The compounds have been identified by comparing their retention times h those of pure compounds on three separate columns. Similar chromatograms were ob-ned for the volatiles of cooked fresh clam meats, fresh raw irradiated clam meats, and e-day-old raw clam meats. When the chromatograms were compared, it was found that adiation at 350,000 rads and storage for 9 days affected carbonyl concentrations similarly.

Both variables caused large increases in peaks 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15 and 16; a slight increase in peak 14; and no peaks were lost. By large increases, it is meant the peak areas were increased by a factor of five or more, and slight increases by a factor of two or less. Irradiation caused the appearance of 2 (possibly as much as 4) new small peaks.

Cooking caused a large increase in peaks 1, 2, 6, 11, 12, 13, and 15; moderate increases in 14 and 15; large decreases in 3, 4, and 7; a slight decrease in 9; and peak 5 was lost.

It is possible that carbonyl compounds may exist in quantities too small to detect which may account for the apparent appearance and disappearance of peaks in the chromatograms when the volume of a compound passes beyond the threshold of detectability.

The determinations of total carbonyls are presently being continued as a secondary quantitative method. It has value as a check on the results obtained with the gas chromatographic method.

Results in recent experiments indicate that the detectable volatile carbonyls disappear as the time of storage increases beyond 30 days, and that irradiation reduces the times required for carbonyls to reach their maximum concentration and their ultimate loss.

Sulfide compounds were studied by two separate methods:

(1) A colorimetric method (1955) involving the production of methylene blue.

(2) Sulfides were also studied using a modification of the Bassette method (1962). Gas chromatographic analyses for sulfides permitted identification of the individual compounds by comparing their retention times with retention times of known sulfides.

Briefly, the Bassette method involves separating a sample into two parts. One part is reacted with mercuric chloride which removes the sulfides from the sample mixture. When the two aliquots are individually chromatographed, the sulfide compounds in the unreacted aliquot can be determined by noting which of the peaks are missing in the chromatogram of the reacted sample.

Tentative identification of the unknown sulfides was made by the method of Baumann and Olund (1962) which related relative retention times of the compounds to their molecular weights.

The details of these experiments will appear in a series of papers which are presently being prepared for publication by staff members of the Gloucester Technological Laboratory.

Employing the quantitative methylene blue method, it was found that total sulfides in clam meats increase with storage. The irradiated samples did not show as rapid an increase in sulfides as did the unirradiated samples. Besides permitting one to follow the overall change in total sulfides, this method also has value as a check on the results obtained by the gas chromatographic method. However, the method does not permit identification of the individual compounds.

Using the modified bassette gas chromatographic technique, five compounds have been tentatively identified. They are hydrogen sulfide, dimethyl sulfide, dimethyl disulfide, methyl mercaptan, and ethyl isopropyl sulfide.

<u>PRE- AND POST-IRRADIATION STUDIES</u>: Although it was necessary to use extremely fresh fish for the optimum dose determination studies, it was recognized that in commercial practice the radiation process would have to be applicable to fish one week or more out of the water. A project was undertaken to show the effects of the pre-irradiation quality of the product on its post-irradiation quality and shelf life.

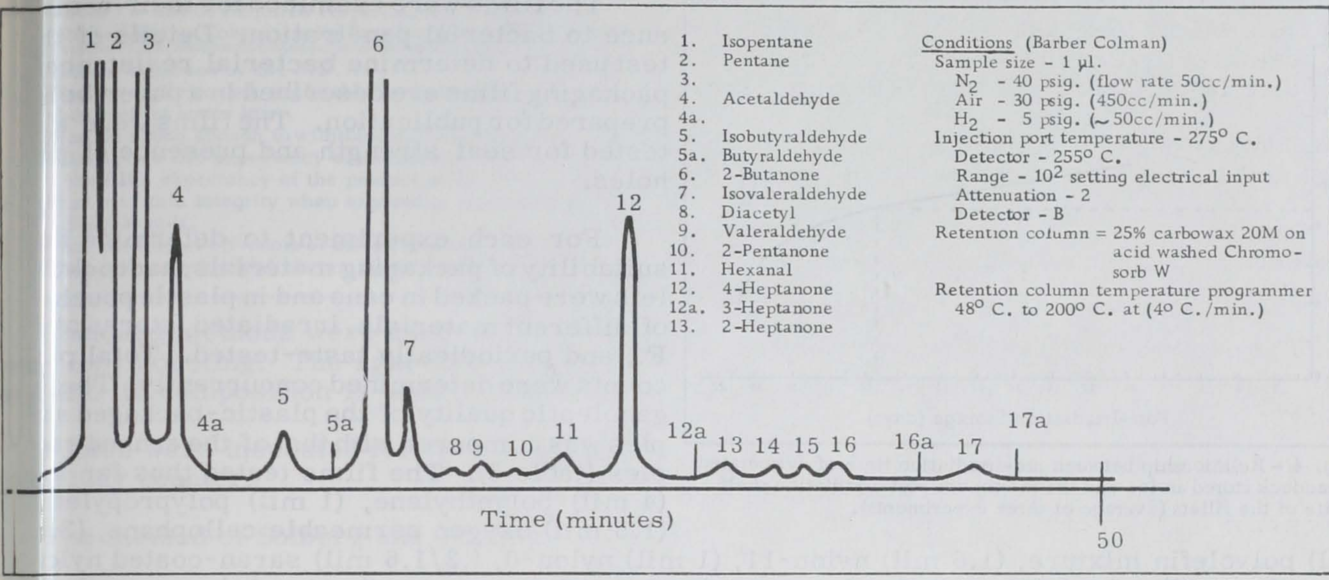

Fig. 2 - Gas chromatogram of concentrated volatile carbonyl compounds in raw, unirradiated clam meats held for two days at 34° F.

Table 4 - The Post-Irradiation Shelf Life at 34° F. of Air-Packed Skinless Fillets Cut from Haddock Iced for Different Periods Prior to Irradiation

Pre-Irradiation Iced Storage	Maximum Post-Irradiation Shelf Life			
	1st Exp.	2nd Exp.	3rd Exp.	Avg.
Days	. . . . (Days)			
4	26	33	33	31
7	16	32	35	29
9	13	27	30	23
11	10	10	16	12
16 (spoiled)				

The very freshest haddock and haddock fillets(haddock was the first species studied) were brought into the laboratory and the fillets were immediately plate frozen at -40° F. and stored at -5° F. The fish were iced in large wooden boxes to simulate shipboard holding conditions. Periodically, some fish were withdrawn and filleted, and the fillets were canned and irradiated at the optimum dose and stored at 34° F. The fillets were organoleptically tested periodically until the onset of spoilage.

In the last haddock experiment, the samples were also analyzed for total plate counts.

The results of those experiments are shown in table 4 and figure 2.

The curve in figure 2 represents the average of the data obtained in three separate experiments which are shown in table 4. From the curve it can be seen that when fish are held in ice for not longer than about nine days, a considerable post-irradiation shelf life can be expected. However, as the pre-irradiation time is increased beyond nine days the post-irradiation shelf life is sharply reduced.

The difference in maximum post-irradiation values obtained in the three experiments shown in table 4 reflect differences in the original quality of the fish in each batch.

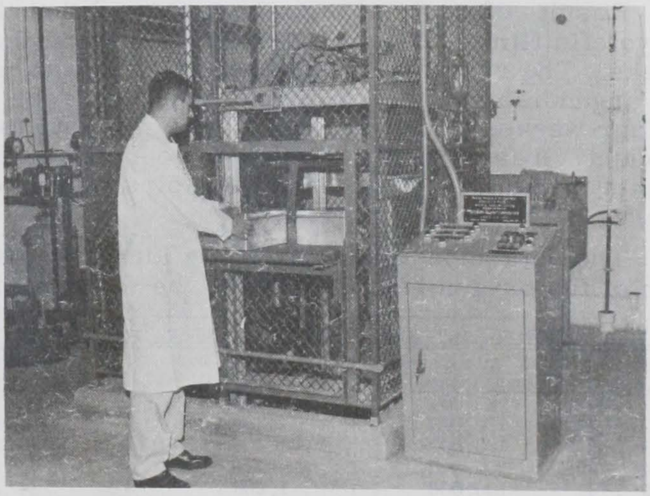

Fig. 3 - Loading 30-pound fillet tins on fast conveyor. Product will be transported through maze into the radiation cell for pasteurization.

PACKAGING STUDIES: Because of the many advantages offered by flexible packaging materials, a study was initiated to determine the suitability of the available plastics to hold irradiated fish and shellfish.

Several films were chosen for study according to desirable properties as shown in table 7.

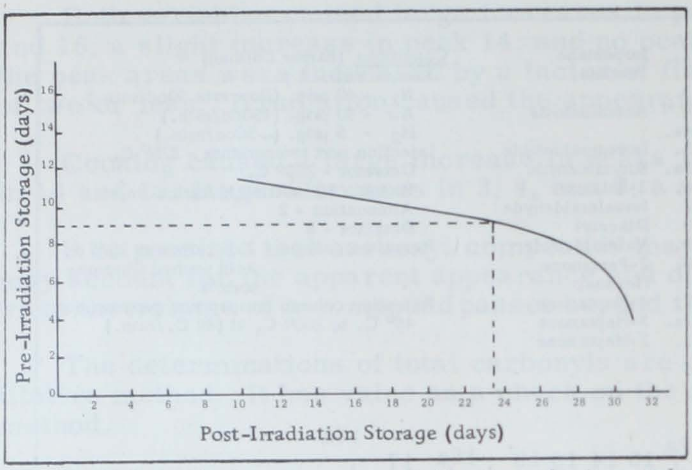

Fig. 4 - Relationship between pre-irradiation time of eviscerated haddock stored in ice and the maximum post-irradiation shelf life of the fillets (average of three experiments).

The films were examined for their resistance to bacterial penetration. Details of the test used to determine bacterial resistance of packaging films are described in a paper being prepared for publication. The films were also tested for seal strength and presence of pinholes.

For each experiment to determine the suitability of packaging materials, haddock fillets were packed in cans and in plastic pouches of different materials, irradiated, stored at 3 F., and periodically taste-tested. Total plate counts were determined concurrently. The organoleptic quality of the plastic-packaged samples was compared with that of the canned samples (table 5). The films tested thus far are (4 mil) polyethylene, (1 mil) polypropylene, (1.5 mil) oxygen permeable cellophane, (3.5) mil) polyolefin mixture, (1.6 mil) nylon-11, (1 mil) nylon-6, (.2/1.6 mil) saran-coated nylon-11, and (2 mil) polyolefin-coated polyester. A publication covering that work is presently being prepared.

All of the films tested were found to be impermeable to bacterial penetration under the conditions to which they were exposed. There is no recognized method for determining bacterial permeability of plastic materials; in fact, there is practically no published work available on the subject. The original method used for this work is being developed further and will be published as a suitable and reliable technique for determining the bacterial permeability of plastic packaging materials.

Table 5 - Quality Data of Haddock Fillets Air-Packed in Different Materials, Irradiated with 250,000 Rads, and Stored One Month at 34° F.

Packaging Material	Average Organoleptic Scores[1]			Total Plate Counts/g ($x10^6$)		
	Experiment No.			Experiment No.		
	1	2	3	1	2	3
Nylon-11	2.0	2.2	2.3	94.0	1.6	12.0
Saran-coated Nylon-11	2.0	2.1	2.7	1.1	61.0	17.0
Polyolefin-coated polyester	2.2	2.0	2.2	17.0	18.0	3.0
Metal can	2.1	2.4	2.3	1.9	24.0	2.8

1/A 5-point scale was used where: 5 - excellent, 4 - very good, 3 - good, 2 - fair, 1 - poor.

The films were found to be relatively free from pinholes and of sufficient seal strength.

Results of early experiments showed that the polyethylene, polypropylene, and the mixed polyolefin films did not protect the quality of the product for periods longer than two to three weeks. The results of later experiments with nylon-11 and polyester are shown in table 5, and the organoleptic data clearly indicate that those films were as suitable as the can for a period of four weeks. However, it will be noted that the bacterial numbers were lower in the canned product. It is expected that if the products were held for a longer period, the higher total plate counts in the plastic-packaged samples would be reflected in significantly lower organoleptic scores.

The data appear to support the idea that the quality loss in the stored product is a function of the oxygen permeability of the material in which it is contained. This is particularly true for pasteurized products with a surviving flora which has at least a partial dependence on oxygen.

Table 6 - Bacterial Reduction in Haddock Fillets by Irradiation at 250,000 Rads

Sample No.	Total Plate Counts/g Prior to Irradiation	Total Plate Counts/g After Irradiation	Percentage Reduction in Total Plate Count
1	83,000	450	99.5
2	170,000	200	99.9
3	180,000	450	99.7
4	100,000	990	99.0
5	100,000	1,200	99.0
6	1,300,000	2,200	99.8
7	5,500	50	99.1

Note: Values are averages of duplicates.

MICROBIOLOGY: Routinely whenever a batch of fish was brought into the laboratory at the start of a storage study, initial total plate counts were made. Total plate counts were also made after irradiation to determine the reduction in bacteria as a result of the process and some of the data obtained are shown in table 6.

Table 7 - List of Required Properties for Packaging Materials to be Used as Containers for Irradiated Fish and Shellfish

Must be approved by the F.D.A.
Must be easily sealed, preferably by ordinary heat-sealing methods.
Must prevent water loss.
Must be strong enough to withstand moderate handling conditions.
Must have a life expectancy of at least two months at 32° F. (The shelf life expectancy of the product at 33° F. is 1-2 months.)
Must maintain integrity when exposed to irradiation doses of up to 500,000 rads.
Must not impart off-odors or off-flavors to the product.
Must prevent microbial contamination of the product.

Table 8 - Formula of Agar	
	g/1
BBL trypticase	15.0
Phytone	5.0
Sodium chloride	4.0
Sodium sulphite	0.2
L. cystine	0.7
Dextrose	5.5
Bacto yeast agar	5.0
Agar	15.0
Final pH 7.0 ± 0.1.	
Sterlized in autoclave @ 118° C. for 15 minutes.	

Note: This formula was developed by Professor J.T.R. Nickerson of M.I.T.

Standard methods were used in sampling, plating, colony counting. The agar was a modified Eugon and its composition is shown in table 8.

Plates were incubated for five days at 20° C. and the colonies were counted using a Quebec colony counter.

The method of Solberg and Proctor (1960) was used for distinguishing small colonies.

Table 6 lists some of the total plate count data obtained from haddock fillet samples, and that species a radiation dose of 250,000 rads apparently reduced the bacterial numbers by at least 99 percent.

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