

Preservation of Fish by Irradiation

Proceedings of a Panel, Vienna, 15-19 December 1969
organized by the
Joint FAO/IAEA Division
of Atomic Energy
in Food
and Agriculture



INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1970

PRESERVATION OF FISH BY IRRADIATION

PANEL PROCEEDINGS SERIES

PRESERVATION OF FISH BY IRRADIATION

PROCEEDINGS OF A PANEL
ON THE IRRADIATION PRESERVATION
OF FOODS OF MARINE ORIGIN
ORGANIZED BY THE
JOINT FAO/IAEA DIVISION OF ATOMIC ENERGY
IN FOOD AND AGRICULTURE
AND HELD IN VIENNA, AUSTRIA
15 — 19 DECEMBER 1969

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1970

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IAEA, VIENNA, 1970
STI/PUB/196

Printed by the IAEA in Austria
November 1970

FOREWORD

The increasing emphasis on the need for protein-rich foods to improve the nutritional status of the world's population has stimulated research on new methods of preserving fishery products. The sea offers a source of protein that could markedly reduce the protein gap existing in many parts of the world. The major problem in attaining a more complete exploitation of these products is how to preserve them so that they can be transported from the points of origin to areas of greatest need. The use of ionizing radiation as a preservation method has been satisfactorily demonstrated in many countries. In some respects it offers advantages over conventional methods; for example, it prolongs the market life without using heat or chemicals, and what is more, the products are treated while they are sealed in commercial types of containers.

Although radiation processing appears feasible, additional research is needed to improve certain quality attributes of some treated foods and to demonstrate to the satisfaction of health authorities that no deleterious effects result. To obtain advice on the problems that need attention a Panel on Irradiation Preservation of Foods of Marine Origin was convened in Vienna, by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, on 15–19 December 1969. The main purpose of the panel's work was to advise the Director General of the Food and Agriculture Organization of the United Nations and the Director General of the International Atomic Energy Agency on how these organizations can best fulfil their roles in radiation research on fishery products. The meeting was attended by thirteen experts from various countries and one representative of the World Health Organization.

Reports were presented on research performed and detailed discussions were held on work done in perfecting the radurization treatment of fishery products. Evaluations were made of the microbiology of the radurization process, with special reference to the control of Clostridium botulinum, type E, in irradiated products.

Recommendations were made on the research needed to solve specific problems and to obtain the information necessary for a full understanding of the mechanisms involved in certain biological systems that bring about the spoilage and deterioration that decreases the market life and usefulness of fishery products.

The proceedings contain the contributions of the members of the panel with the general conclusions and recommendations for further research.

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STUDIES IN PETITION-ORIENTED ASPECTS OF RADIATION PASTEURIZATION OF FISHERY PRODUCTS

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Abstract

STUDIES IN PETITION-ORIENTED ASPECTS OF RADIATION PASTEURIZATION OF FISHERY PRODUCTS.

Following the many successful experiments substantiating the feasibility of preserving fishery products with gamma rays, there remains the task of obtaining approval from the U.S. Food and Drug Administration before the process can be used in commerce. The Gloucester Technological Laboratory has conducted research in two pertinent areas: in one study an empirical test has been used to collect data in one aspect of a multi-laboratory investigation of a possible botulism hazard in radiation-pasteurized fishery products; in the second study the efficacy of the process was substantiated under commercial shipping conditions.

1. INTRODUCTION

Early research conducted at the Massachusetts Institute of Technology (Cambridge, Mass., USA) indicated the feasibility of preserving marine products with low levels of ionizing radiation (Proctor et al. 1953 [1], Proctor et al. 1960 [2], and Nickerson et al. 1960 [3]). Numerous research teams have investigated and expanded on the early work conducted by Proctor et al. and Nickerson et al., and, in the United States, the largest effort has been under the "Atoms for Peace" program of the U.S. Atomic Energy Commission (a comprehensive, though not quite up-to-date, listing of the publications resulting from this program appears in Anonymous, 1969 [4].) These researchers were in agreement as to the preservative effects of low levels of ionizing radiations on marine fish and shellfish and on fresh-water fish as well. Their results, which covered the practical aspects of application, indicated the attainment of a significant extension of the product shelf life using radiation-dose levels in the range of 100 to 600 kilorads. Thus, the use of radiation for preserving fishery products has been given a reasonably thorough investigation resulting in promising results.

Since the application of radiation is defined as a food additive under the food-additives amendment to the Federal Food, Drug, and Cosmetic Act of the United States, irradiation cannot be approved for use on foods

unless it meets with the food-additive compliance. Kirk (1966) [5] described the information which must be provided:

- (a) A description of the radiation process.
- (b) The proposed conditions of use.
- (c) Data establishing that the radiation accomplishes the intended physical or other technical effect, and that the proposed amount is not higher than that reasonably necessary to produce the effect.
- (d) Analytical methods to determine the amount of radiation, if any present (where needed).
- (e) Investigations made with respect to the safety for use of the radiation including complete information about the methods and controls used in conducting the investigations.

In addition, the statute specifically directs, in part, that no such regulations shall issue if a fair evaluation of the data: (a) fails to establish that the proposed use of the food additive under the conditions of use to be specified in the regulation will be safe; provided, that no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or (b) shows that the proposed use of the additive would promote deception of the consumer in violation of this Act or would otherwise result in adulteration, or mis-branding of food within the meaning of this Act.

The technological laboratories of the Bureau of Commercial Fisheries, U.S. Department of the Interior, have conducted research in co-operation with other research laboratories in the United States of America to obtain the pertinent information required before the radiation process can be approved by the U.S. Food and Drug Administration. This laboratory has recently conducted work in two of the areas considered to be important. One objective was to attempt to resolve as to whether or not there exists a botulism hazard in radio-pasteurized fishery products. The second objective was to carry out simulated commercial-scale shipping studies in order to determine the practical effectiveness of the process as well as to learn something about the minimum required dose to provide the intended effect.

2. TEST FOR BOTULISM HAZARD

Two consequences of pasteurizing fish by radiation have given rise for concern: first, the bulk of the spoilage bacteria (pseudomonads) is highly susceptible to radiation, and it is generally believed that elimination of these bacteria will delay, or possibly eliminate, the development of typical, objectionable compounds normally associated with "spoiled" fish. The second consequence of pasteurizing fish is that to achieve any benefit from pasteurization, the fish must be contained in a package to prevent recontamination. Thus, in the absence of the spoilage bacteria, and in the confines of a package, the possibility that the spores (some of which survive pasteurization) of anaerobic

pathogens will vegetate and produce toxin is presumably greatly enhanced should the product be mishandled. The fact that irradiated fish does eventually spoil has already been resolved (Slavin et al. 1966 [6]). There remained the task of resolving whether or not there is a botulism hazard in preserving fish by irradiation.

A test was, therefore, developed for evaluating whether or not there exists a botulinum Type E hazard in fish fillets pasteurized with gamma radiation. The method is based on a comparison of "safety margins" (the time interval between spoilage and toxin production) in inoculated and uninoculated irradiated and non-irradiated fillets. The procedure involves three parts: (1) measure the maximum shelf life, or its equivalent, of radio-pasteurized and non-irradiated fish fillets; (2) obtain the time required for threshold-level toxin to develop in radio-pasteurized and non-irradiated fish fillets; and (3) make critical analyses of the results obtained in (1) and (2) to obtain a measure of the safety margins of both radio-pasteurized and non-irradiated fish fillets.

2.1. Maximum shelf life of the product

This term, now called "X-value" by researchers in the field, is defined as the maximum shelf life of a sample when it is unanimously rejected by a panel of adequate size (20 or more members). A description of the organoleptic methods is found in Learson and Ronsivalli (1969) [7].

The major variables which affect the shelf life of irradiated fish are the storage temperature and the amount of irradiation absorbed. Thus, the samples should be stored at sufficient number of temperatures in order to permit interpolation of the effect to any temperature within the range, and samples should be irradiated at practical radiation levels.

2.2. Toxin development

As we stated earlier, this test is based on a comparison of "safety margins" in radio-pasteurized and non-irradiated samples, and the margin for any sample is the time interval between its maximum shelf life and the time required for toxin to develop.

Under the co-ordination of the Division of Biology and Medicine of the United States Atomic Energy Commission, several researchers have investigated the times required for the development of toxin in haddock fillets (Goldblith and Nickerson 1965 [8]; Segner and Schmidt 1968 [9]; and Eklund and Poysky 1968 [10]).

Haddock fillets were inoculated (at levels of 10^2 , 10^4 , and 10^6 spores per gram) in containers and stored at various refrigeration temperatures. At intervals of storage, the inoculated samples were tested for the presence of toxin by the mouse-injection method.

2.3. Analysis of data

For the problems of analysis and interpretation, the graphs measuring spoilage time and toxin formation are remarkably similar. In each case the picture is that of a sigmoid-shaped curve, of the type shown in Fig. 1. Such curves lend themselves to probit analysis (Finney 1952 [11]). This type of analysis, commonly used for analysing pharmacological data explicitly investigates the normality of the distribution and only draws conclusions when agreement with normality has been established. Thus, development of spoilage (as measured by the percentage rejection of the organoleptic panel) and toxin accumulation (as measured by the mouse-injection method) may, in each case, be studied by conversion of the ordinates to the probit scale. By this method the sigmoid curves represented by the data are converted to straight lines. In this way the "tails" of the original time studies become part of their linear mid-portions.

The importance of this change becomes evident when it is considered that the original sigmoid curves are notably difficult to assess with reliability at the extremes (i.e. at the tails). Hence the maximum shelf life which required 100 per cent rejection by the panel for its determination is precisely at that part of the curve where confidence intervals are wide, precision is low, and crucial decisions as to the precise time of spoilage will be difficult to make.

The relationship between spoilage time and toxin-development time may be given its clearest statement in terms of these calculated RT_{50} and TD_{50} averages, since these are most reliable (have the narrowest confidence bands). In this way, the 50 per cent rejection point can be determined with great precision and the 100 per cent rejection time can be estimated by taking the RT_{50} plus 3 or 4 standard deviations.

Each member of an untrained consumer panel is studied for his judgement of spoilage against the average judgement of the trained panel. The day on which the consumer panelist reports spoilage is compared to the average day of rejection by the trained panel for this sample of fish. The comparative times of spoilage are then plotted.

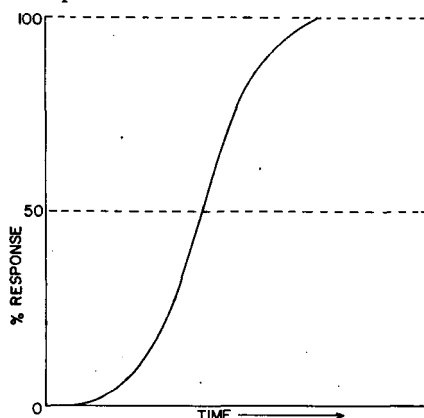


FIG. 1. A typical biological-response curve.

Consumers studied in this way to date have shown no systematic shift of sensitivity in one direction or another with level of irradiation or temperature of storage. That is, sensitivity or lack of it, elevated or depressed threshold, exists rather uniformly independent of temperature or radiation effect.

With the tools of probit analysis and regression analysis, the basic requirements for estimating the safety factor in radio-pasteurization with regard to botulism, and the further identification of the true consumer population at risk, are provided. Currently, investigations are under way applying these quantitative methods on a nation-wide basis. In this way, factors of geography, climate, and lot variability will be considered to arrive finally at a reasoned decision as to whether or not a botulinum hazard exists in radio-pasteurization of fish products.

3. SIMULATED COMMERCIAL-BENEFIT SHIPPING STUDIES

To permit the necessary demonstrations of the commercial-scale feasibility of radiation preservation, the Food and Drug Administration authorized this laboratory to engage in large-scale shipments of radiation-treated fish fillets (Stevens 1965 [12]). These commercial-benefit shipping experiments were conducted under carefully controlled conditions to prevent any possibility of the irradiated food entering retail-food channels.

During 1965, we collected data on the temperature patterns occurring in fresh fillets as normally shipped in interstate commerce by common carriers, and these data indicated that present commercial shipping practices are more than adequate to ensure the safe distribution of irradiated fillets. The FDA-sanctioned benefits-evaluation study of irradiated and non-irradiated fillets was then initiated and carried out under commercial conditions.

Three experiments, each involving shipments of fillets of cod and haddock, irradiated at dose levels of 0, 100 and 200 kilorads, at the averaged points of minimal absorption, were conducted.

3.1. Procedure

Fillets of cod and haddock were purchased on the open market, irradiated, placed aboard commercial interstate carriers and sent to locations far beyond the present geographical limits of the North-eastern fresh-fish market (see Fig. 2). For each shipment, the test fillets were separated into two sets. One set, consisting of both irradiated and non-irradiated fillets was retained as a control and evaluated at the Bureau of Commercial Fisheries Technological Laboratory, Gloucester, Massachusetts throughout the test period. The other set, again consisting of irradiated and non-irradiated fillets, was used for the commercial shipments. A Bureau of Commercial Fisheries technologist travelled with each of the shipments to monitor shipping temperatures, to ensure that the irradiated products did not get into retail outlets and, at the destination point, to ship the test products back by air to Gloucester for testing.

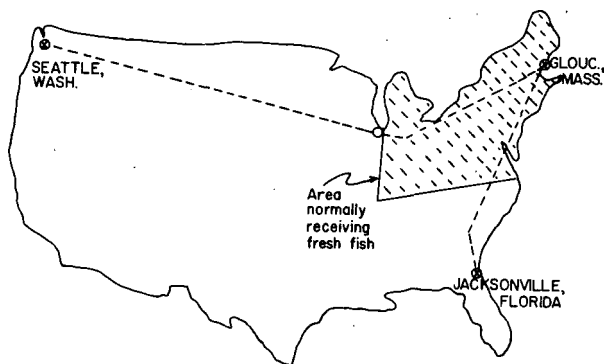


FIG. 2. Cities involved in shipping studies.

Two factors must be considered in assessing the commercial benefits-evaluation tests. The first is that the product is maintained at low temperatures in commercial channels of distribution, regardless of the season of the year. The second is that the fillets used in the three shipments discussed here were, unavoidably, of mixed quality.

An example of the three long-distance benefits-evaluation shipments may be found in the Seattle shipment of 23 February 1967. Commercially available fillets of haddock and cod were purchased on the open market on 21 February and irradiated in 10-pound fillet cans on 22 February 1967. The irradiated fillets and non-irradiated controls, after withdrawal of samples to be held in the laboratory, were randomly placed in barrels (ten 10-pound fillet cans per barrel), iced under normal conditions (1.5 parts of ice to 1.0 part of fish) and shipped via the Railway Express Agency from South Station, Boston, Massachusetts late in the evening of 23 February 1967.

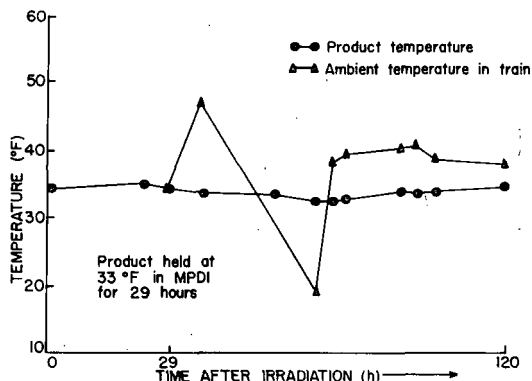


FIG. 3. Temperatures of product during shipment to Seattle.

Bureau of Commercial Fisheries Nomenclature			Food and Drug Administration Nomenclature	
Hedonic scale	Odour	Flavour	Classification	Odour
9 Excellent	Sea-fresh fresh odour characteristic of species	Neutral, faint sweet flavour characteristic of species	0	Practically no odour
8 Very good	Faint sea-fresh odour	Increase of sweet and characteristic flavour of species		
7 Good	Neutral or no odour	Decrease of sweet and characteristic flavour		
6 Fair	Very slightly fishy	Loss of flavour, very slightly fishy or musty	1	Almost complete lack of odour or only a slight "fishy" odour
5 Borderline	Slightly fishy	Neutral or slightly fishy		
4 Slightly poor	Strongly fishy, fleeting NH_3	Fishy or incipient spoilage	2	Odour not intense but persistent and readily perceptible to the experienced examiner who recognizes it as indicative of decomposition
3 Poor	Slight but persistent NH_3 fruity, sour, oniony or slightly yeasty	Strongly fish, sour acid, sulphide, slight but persistent NH_3		
2 Very poor	Stale or "rancid" strong NH_3 , moderately yeasty	Salty, bitter, sulphide, strong NH_3	3	As 2 above but readily noticeable and is indicative of decomposition so advanced as to be distinct and unmistakable
1 Inedible	Strongly yeasty, nauseating, putrid faecal, indole, H_2S excessive NH_3 , revolting or sickly sweet	Nauseating, revolting, bitter, sulphide or excessive NH_3		

FIG.4. Comparative descriptive nomenclature, organoleptic evaluation of cod and haddock fillets.

Transfers of the samples between trains were made twice, once in Chicago and once in St. Paul, Minnesota. Owing to the seasonably cold weather, no re-icing of the barrels was necessary from coast to coast. Figure 3 shows the temperature profile of the product during this shipment. The shipment arrived in Seattle, Washington early in the morning of 27 February 1967, five days after irradiation, and was delivered by the Railway Express Agency to the Bureau of Commercial Fisheries' Seattle Technological Laboratory later the same morning. The shipment was then divided in two portions, one to be kept in Seattle for evaluation by the Seattle laboratory and the other to be shipped by air-freight back to the Gloucester Technological Laboratory. The return shipment left Seattle on 28 February by air and was delivered in Gloucester on 1 March 1967, eight days after irradiation.

The organoleptic procedures used for this report involved evaluation as to appearance, odour, flavour, and texture by a panel of 12 members trained in such evaluation on normal commercially available fish fillets. The evaluation system used was a modified hedonic system using a score of from 0 to 9 and providing space for separate evaluation as to appearance, odour, flavour, and texture. This modified hedonic scale was related quantitatively to specifically defined quality attributes and to the FDA system for organoleptic evaluation of fish fillets (see Fig. 4).

TABLE I. TOTAL PLATE COUNTS IN SAMPLES USED IN SHIPPING EXPERIMENTS (VIA RAILROAD)

Experiment No.	Destination	TOTAL PLATE COUNT							
		Before shipping				Upon return to MPDI			
		Date	Control	100 krad	200 krad	Date	Control	100 krad	200 krad
1	Jacksonville	13/2/67	1.2×10^5	3.4×10^3	1.7×10^3	16/2/67	4.6×10^5	1.6×10^5	2.5×10^3
			4.0×10^5	4.9×10^4	4.3×10^3		1.6×10^7	2.3×10^6	6.2×10^4
2	Seattle	22/2/67	1.6×10^5	1.4×10^4	2.3×10^3	1/3/67	3.1×10^6	6.2×10^6	3.2×10^4
			2.0×10^5	2.4×10^4	6.8×10^3		8.8×10^6	5.0×10^6	5.4×10^5
3	Jacksonville	6/3/67	1.1×10^5	5.6×10^3	1.4×10^3	9/3/67	1.3×10^7	4.0×10^5	6.0×10^3
			1.1×10^5	7.0×10^3	3.1×10^3		3.8×10^6	4.0×10^5	1.8×10^4

3.2. Results

Table I summarizes the bacterial profile of the samples of the three shipping experiments. It shows reduction, at the 200-kilorad treatment level, of at least 2 log cycles of bacterial numbers under commercial conditions of irradiation and shipment to points more than double in distance than those currently being made by industry. The distances and durations involved in these overland shipments are unprecedented for shipping fresh fish, and the benefits to be gained by irradiation of fish fillets becomes obvious.

All of the irradiated fillets in these three shipments were of acceptable quality at all points of examination, including those tested after being returned to the Bureau of Commercial Fisheries Gloucester Laboratory. In contrast, the non-irradiated fillets were spoiled when returned to Gloucester or within one or two days after being returned. The irradiated fillets continued to be of good quality for an additional eight to ten days after being returned to Gloucester. A graphic illustration of the preservative effect of irradiation, in one of the experiments, is shown in Fig. 5.

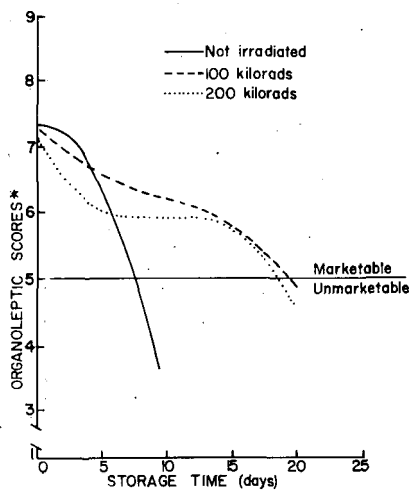


FIG. 5. Shelf life of irradiated and non-irradiated haddock fillets held at 33—35°F.

* 9 = excellent, 8 = very good, 7 = good, 6 = fair, 5 = borderline, 4 = slightly poor, 3 = poor, 2 = very poor, 1 = inedible.

The averages of marketable shelf lives of commercially-handled and processed haddock fillets for the three shipments, as determined by sensory evaluation and computer-analysis, were 7, 15 and 18 days for irradiation levels of 0, 100, 200 kilorads, respectively. The equivalent findings for cod fillets were 6, 16 and 20 days.

Thus, it appears that irradiation will permit maintenance of quality of fish fillets during an extended shelf life which, of course, appeals to the

industry. Producers who know of our work have indicated that irradiation processing will help to smooth out the highs and lows of availability of fresh-fish supplies and will help to ensure a steadier market. Most retailers see no particular advantage in extending the shelf life for much more than 11 to 12 days over that of the non-irradiated fresh fish. Retailers claim that using irradiated seafoods would permit holding of the fillets after the peak demand day in the week has passed rather than having to mark down the price or discard the fillets due to spoilage. The process would enable retailers to offer fresh fish throughout the week to a degree greater than is now possible. Producers also claim that these savings can be passed along to consumers. Spokesmen for eight of the largest chain supermarkets in the nation state that they could and would sell irradiated fresh seafoods in areas where fresh seafoods are not sold now.

4. SUMMARY

Following the many successful experiments substantiating the feasibility of preserving fishery products with gamma rays, there remains the task of obtaining approval from the U.S. Food and Drug Administration before the process can be used in commerce. The Gloucester Technological Laboratory, working with other U.S. laboratories towards a common goal, is conducting research in two areas considered to be essential for obtaining approval of the process. In the first study, an empirical test is being used to attempt to resolve whether or not there is a botulism hazard in radiation-pasteurized fish. The test, based on a statistical evaluation of the difference between the time treated fish undergo discernible spoilage and the time they develop botulinum toxin, is expected to permit a reliable decision regarding the safety of the process. Although some of the data have been obtained, more data are necessary before a decision can be reached. In a second study, experiments were conducted to obtain information on the efficacy of the process under commercial conditions. The results of tests, involving commercial carriers and cross-country shipments, indicate that the process is definitely efficacious and that many economic benefits can accrue to the industry.

ACKNOWLEDGEMENT

This work was sponsored by the Division of Isotopes Development, U.S. Atomic Energy Commission, Washington, D.C.

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THE EFFECT OF GAMMA IRRADIATION ON THE MICROBIAL SPOILAGE PATTERNS ON FISH, IN RELATION TO INITIAL QUALITY

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Abstract

THE EFFECT OF GAMMA IRRADIATION ON THE MICROBIAL SPOILAGE PATTERNS ON FISH, IN RELATION TO INITIAL QUALITY.

The effect of age of fish prior to irradiation (100 krad) on the post-irradiation storage life and bacterial flora of aerobically stored haddock fillets has been investigated. Significant extensions in storage life were found after irradiation, even for fish judged to be of fairly low quality initially. However, fillets from older (low quality) fish were found to be borderline in quality for most of their extended storage life, while fillets from fresh fish were borderline in quality only during the latter half of their extended storage life. Although bacterial numbers were initially lower on irradiated fish, 2-day and 5-day irradiated fish had 10-100 times as many bacteria as their unirradiated counterparts at spoilage. Initial quality did not appear to affect, qualitatively, the flora developing during storage on unirradiated fillets on which pseudomonads and Achromobacter predominated. However, on irradiated fillets, while Achromobacter predominated, a change in the character of the Achromobacter isolates from 2-day and 5-day irradiated fish, was observed at spoilage.

1. INTRODUCTION

Gamma irradiation has been shown to radically alter, both qualitatively and quantitatively, the microbial flora of fish and other seafoods. In general, in temperate latitudes, pseudomonads have been shown to be replaced as the principle component of the bacterial flora by the less biochemically active but more radiation-resistant achromobacters during aerobic refrigerated storage of irradiated fish. The importance of the pseudomonads in producing the characteristic odours which render fish unacceptable to the consumer is widely recognized (Castell et al 1948 [3], 1957 [4,5], 1959 [6]; Lerke et al. 1963 [12], 1965 [13]; Adams et al. 1964 [1]; Shaw and Shewan 1968 [19]). Hence, the reduction or complete elimination of pseudomonads combined with an overall reduction in bacterial numbers can be expected to be accompanied by an increase in storage life. Numerous workers have in fact shown increases in storage life for a variety of seafoods after gamma irradiation. Work in our laboratory by Power et al. (1964 [16, 17], 1967 [18]) has shown radiation to be an effective means of extending the storage life of scallops, lobster and haddock.

Various interacting factors can be expected to affect the storage life of the freshly filleted fish. Among them are handling and storage conditions,

which can be controlled, at least in theory; the nature and amount of the contaminating microflora, which can be partially controlled by good sanitary practice; and the state of the substrate, the fish muscle tissue. This latter factor is itself dependent on a complex of factors including the season of the year, and the length of time the fish has been kept in ice prior to filleting. It is at the time of filleting that freshly exposed, and probably still sterile, muscle tissue receives its initial load of microbial contaminants. The condition of the muscle tissue at this time may be expected to affect the nature and amounts of substrates available for microbial attack which in turn will affect the numbers and kinds of micro-organisms developing on the fillets during storage, i.e. the spoilage patterns. An investigation has therefore been made of the effects of age¹ of fish prior to filleting on subsequent quantitative and qualitative changes in the bacterial flora of irradiated and non-irradiated haddock fillets stored aerobically at 3°C.

2. MATERIALS AND METHODS

The fish used in these experiments were haddock (Melanogrammus aeglefinus) caught by side trawler and filleted, after 2 days, 5 days and 9 days in ice, at local processing plants. Henceforth, they will be referred to as 2-day, 5-day and 9-day fish. They were chosen to represent good, medium and poor quality fish arriving at the plant, and were graded by Department of Fisheries Inspectors as shown in Table I.

Table I. GRADING OF FISH

Days held in ice prior to filleting	Grade	Remarks
2	1	Premium
5	1	Borderline grade 2
9	2	Low grade 2

Thus, in fact, only the 2-day and 5-day fish would have appeared on the fresh fish market and so have been candidates for irradiation. However, it is by no means certain that older fillets are always kept off the fresh fish market and so for the sake of completion they have been included.

After filleting, in a regular processing line, the fillets were sealed singly in 1-mil polyethylene bags prior to transportation back to the laboratory where half were given 100-krad irradiation using the Gammacell 220 designed and built by Atomic Energy of Canada Ltd. and described by Power et al. (1964

1 By age is meant number of days since fish were caught and gutted.

[17]). Immediately after irradiation samples were taken for bacteriological analysis and trimethylamine evaluation. Further bacteriological (quantitative and qualitative) trimethylamine and taste-panel evaluations were made during storage at 3°C.

2.1. Trimethylamine analysis

Trimethylamine analyses were performed as described by Dyer (1945 [8], 1959 [9]).

2.2. Organoleptic analysis

For each taste-panel evaluation two fillets of each treatment plus two frozen control fillets were baked in foil-covered pans at 500°F. Taste panels were composed of ten people. They were asked to assess the coded samples for odour and flavour and results were scored on a ten-point scale on which 4 was taken as the limit of acceptability (based on Canada Dept. Agriculture Publication 1284, 1967).

2.3. Quantitative bacteriological analysis

Five fillets were taken for each evaluation and blended with an equal volume of cold, sterile, 0.1% peptone solution in a 2-gallon blender. A 20-g sub-sample was taken and serially diluted in 0.1% peptone. Three dilutions were plated on trypticase glucose yeast-extract agar with added 0.5% peptone and 0.5% sodium chloride, three replicates being plated at each dilution. Plates were counted after 5 days at 20°C.

2.4. Qualitative bacteriological analysis

Organisms to be identified were systematically isolated from dilution plates. Where possible 100 isolates were taken from each treatment. Isolates were characterized using the following tests: Gram stain; morphology; pigmentation; oxidase activity; metabolism of glucose (Hugh and Leifsen 1953 [10]) and arginine (Thornley 1960 [24]); sensitivity to penicillin, 2.5 iu/ml and oxytetracycline, 10 µg/ml; growth on MacConkey agar, on phenyl ethyl alcohol agar and on Staphylococcus 110 medium; gelatin liquefaction and production of trimethylamine from trimethylamine oxide.

3. RESULTS AND DISCUSSION

3.1. Organoleptic assessment

Although this investigation was primarily a bacteriological one it was considered necessary to determine also other closely linked criteria of spoilage. One of these was the assessment of organoleptic spoilage which, although it has many difficulties associated with it, is still, practically, the best means of determining the point in storage at which the fish is spoiled. The other criterion of spoilage evaluated was trimethylamine production which although widely used in practice is perhaps of rather dubious significance.

2-day fish. The storage life of unirradiated 2-day fish fillets was found to be about 9 days at 3°C. Irradiation (100 krad) extended the storage life by 8-9 days, the fillets being unacceptable after 18 days.

5-day fish. Although judged to be of lower quality in the round, fillets from 5-day fish had similar storage lives to those from 2-day fish after irradiation. Unirradiated fillets were unacceptable after 7 days and irradiated fillets after about 18 days.

9-day fish. The fillets from unirradiated 9-day fish had storage lives of about 5 days, radiation extended the storage lives to 13 days.

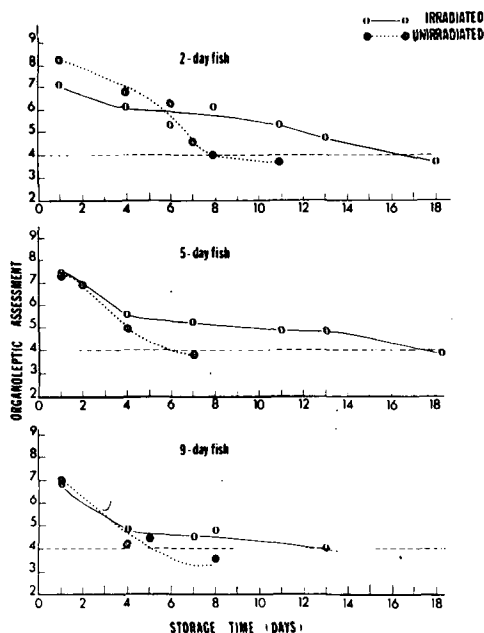


FIG.1. Taste panel evaluations (odour) during storage at 3°C.

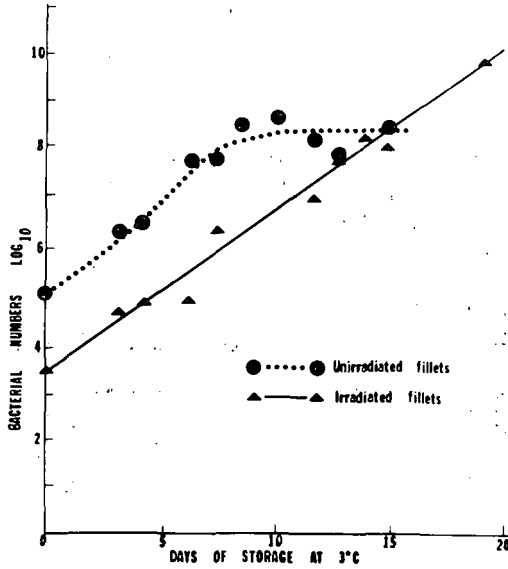


FIG. 2a. Change in bacterial numbers during storage of 2-day fish.

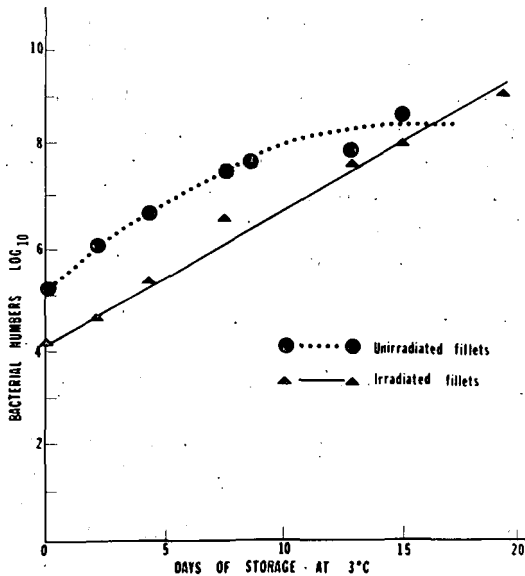


FIG. 2b. Change in bacterial numbers during storage of 5-day fish.

The results just described show significant extensions of storage life after irradiation even for fish judged to be of low quality on landing. On closer examination of the taste panel data, of which Fig. 1 is typical, it became apparent that while the unirradiated fillets tended to deteriorate in quality

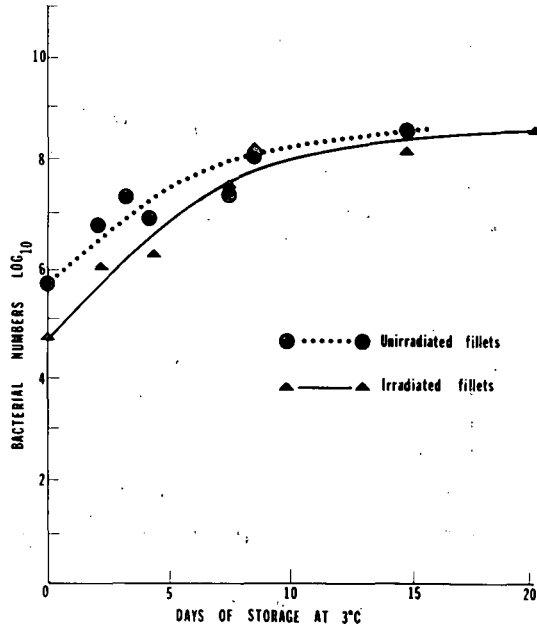


FIG. 2c. Change in bacterial numbers during storage of 9-day fish.

fairly rapidly immediately prior to becoming unacceptable the irradiated samples appeared to remain at borderline quality a long time. A fish is judged unacceptable on the scale used at the level 4 and fish between the levels of 5 and 4 may be considered borderline in quality. On this basis irradiated 2-day fish were borderline in quality for 45-50% of their extended storage life, 5-day fish for 70% of their extended storage life, and 9-day fish for 90% of their extended storage life.

3.2. Quantitative analyses of the bacterial flora (Figs 2 a-c)

Bacterial numbers were found to be of the order of 10^5 per gram on 2- and 5-day fish and around 10^6 per gram on 9-day samples. Irradiation brought about a reduction in numbers of between 1 and 2 log orders. Occasionally a survival rate of greater than 10% was encountered, which was associated with the presence of the genus *Bacillus*. Bacterial numbers were between 10^7 — 10^8 per gram in the unirradiated fish when they were judged spoiled organoleptically and levelled off at around 10^8 per gram. In irradiated 2-day and 5-day fish bacterial numbers increased logarithmically throughout the storage period and were of the order of 10^9 per gram at spoilage. Irradiated 9-day fillets showed an initial logarithmic increase in bacterial numbers from 10^5 to 10^8 per gram after which numbers levelled off, the samples not being judged spoiled organoleptically until some 4 days later. Trimethylamine

evaluations made on the samples used for bacteriological analysis showed that irradiation markedly inhibited the production of trimethylamine during storage of 2-day and 5-day samples, but to a much lesser extent in 9-day samples.

3.3. Qualitative analysis of the bacterial flora (Figs 3, 4 and 5)

The taxonomy of the bacteria associated with marine organisms is at present in a state of flux and this is to some extent reflected in the various levels at which bacteria were identified in this investigation. Four genera of gram-negative organisms were recognized, Achromobacter, Pseudomonas, Flavobacterium and Vibrio. All achromobacters were non-motile coccoid rods or cocci and as such would be classed as Acinetobacter (phenon IV) in Thornley's (1967 [25]) revised version of the Brisou and Prévot (1954 [2]) reclassification of this group of organisms. The pseudomonads were identified mainly on arginine utilization (Thornley 1960 [24]) and glucose metabolism (Hugh and Leifsen 1953 [10]) and grouped according to Shewan et al. (1960 [20]). One large group of brown pigmented, non-glucose oxidizing and non-arginine utilizing organisms was identified as Pseudomonas putrefaciens on

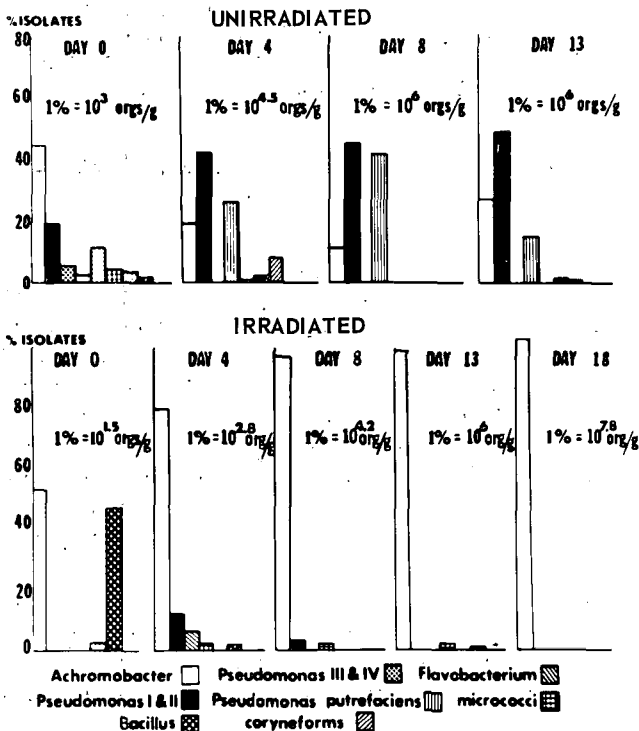


FIG.3. Percentage composition of the bacterial flora of 2-day fish during storage at 3°C.

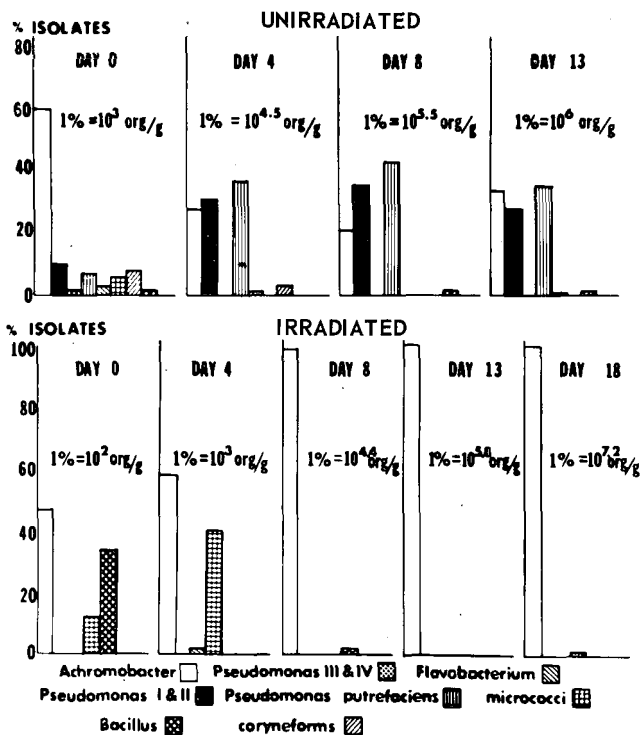


FIG.4. Percentage composition of the bacterial flora of 5-day fish during storage at 3°C.

the basis of H_2S production (Levin 1968 [14]) and flagellation. Flavobacteria were characterized by their morphology, production of yellow or orange pigments and their lack of utilization of arginine. Vibrios, which were only rarely encountered, were separated from pseudomonads by their fermentative action on glucose. Gram-positive isolates were divided, mainly on morphology, into three groups, micrococci, coryneforms and *Bacillus* spp. Micrococci and coryneforms were not identified further save that a group of coryneforms producing a diffusible red fluorescent pigment on King, Ward and Raney's medium [11] could be distinguished. *Bacillus* isolates were nearly all found to be *Bacillus subtilis*.

The bacterial flora of the freshly cut fillet prior to irradiation was found to be varied. Gram-negative organisms predominated, comprising at least 80% of the isolates. The majority of these isolates were achromobacters and pseudomonads, with smaller numbers of flavobacteria and an occasional vibrio. Gram-positive organisms were less frequently isolated although one or more of the groups was always encountered. The number of days the fish had been held in ice prior to filleting did not appear to bear any relation to the relative

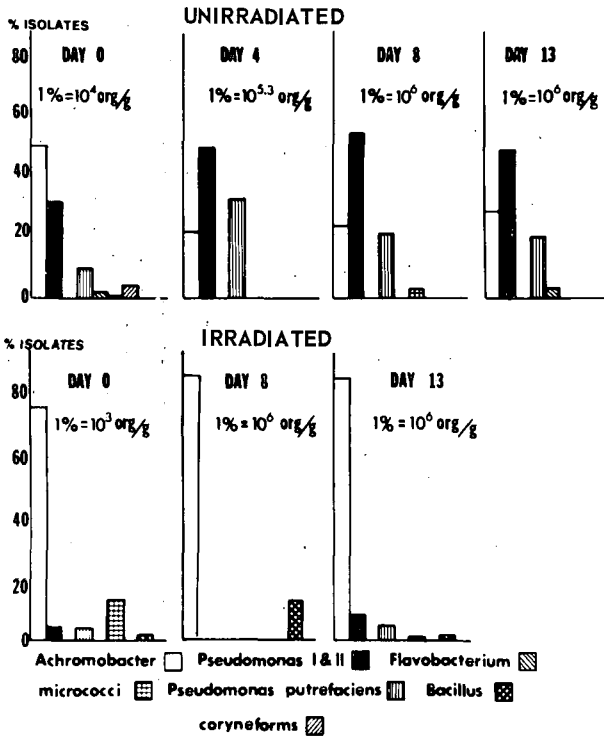


FIG:5. Percentage composition of the bacterial flora of 9-day fish during storage at 3°C.

proportions of Achromobacter, Pseudomonas I and II or gram-positive organisms isolated immediately after filleting. However, the proportions of Pseudomonas III and IV and Flavobacterium were consistently found to decrease with increased storage in ice prior to filleting. Pseudomonas putrefaciens on the other hand was found to increase with the length of time the fish was stored in ice prior to filleting and was generally found to be at a higher level than that found by Chai et al. (1968 [7]) for recently processed haddock fillets.

100-krad irradiation brought about a considerable change in the bacterial flora. Among the Gram-negative organisms Achromobacter formed the major proportion of survivors, although a small porportion of Pseudomonas I and II and Pseudomonas putrefaciens were isolated from 5-day and 9-day fish. The Gram-positive organisms which formed only a small percentage of the pre-irradiation flora enjoyed a selective advantage on irradiation and in some cases were found to form a large proportion of the immediate post-irradiation flora. However, none of them appeared able to compete with even the depleted numbers of Gram-negative psychrophiles and they were eventually outgrown during refrigerated storage.

Three groups of organisms predominated during refrigerated storage of all the unirradiated fillets, Achromobacter, Pseudomonas I and II and Pseudomonas putrefaciens. Achromobacters, although they increased in numbers were found to decrease in proportion of isolates until the time of organoleptic spoilage. After spoilage the percentage of achromobacters among the isolates increased as numbers of the faster growing pseudomonads levelled off. The role of Achromobacter in fish spoilage is not clear. The percentage of Achromobacter isolates capable of producing trimethylamine from trimethylamine oxide decreased during storage at 3°C from 55% immediately after filleting to about 35% at spoilage in both 2-day and 5-day samples (Fig. 6). Proteolytic activity, as assessed by ability to hydrolyse gelatin, was uncommon and when present only very weak. One way in which achromobacters may contribute to spoilage is by production of textural changes. Walker and Jay (1969 [26]) studying bacterial spoilage during the low-temperature storage of irradiated beef showed, using the electron microscope, that bacteria producing slime were able to physically disrupt the organization of the muscle fibrils without any concomitant proteolysis.

Pseudomonas groups I and II increased rapidly until spoilage with the fluorescent group I being outnumbered by the non-fluorescent group II throughout. Only a very small percentage of those isolated were trimethylamine producers or gelatin hydrolysers, although several workers have shown Pseudomonas to be capable of producing spoilage odours in fish (Castell and co-workers 1948 [3], 1957 [4,5], 1959 [6]; Lerke et al. 1965 [13]; Shaw and Shewan 1968 [19]).

Pseudomonas putrefaciens was the most rapidly growing organism isolated during storage. All isolates were strongly proteolytic and reduced trimethylamine oxide. This organism has been shown by Castell et al. (1948 [3]) and by Levin (1968 [14]) to be a major spoilage species on cod and haddock.

Achromobacters were the dominant organisms during the storage of all irradiated fish. The Gram-positive organisms isolated initially were eventually outgrown at the low storage temperature. Most of the Achromobacter isolates in unirradiated fish and up to 13 days of storage in the irradiated fish (when they formed nearly 100% of all isolates) were oxidase-positive and sensitive to penicillin and oxytetracycline. At this stage the 9-day fish were spoiled and no further isolates were made from them. Further isolations were made from 2-day and 5-day irradiated fish at the time of their spoilage, 18 days. By this stage there appeared to have been a great change in the nature of the Achromobacter population. In 2-day fish 67% of the isolates were oxidase-negative, penicillin- and oxytetracycline-insensitive achromobacters, while the remaining 33% were oxidase-positive and oxytetracycline-sensitive, and all but two, penicillin-sensitive achromobacters. In 5-day fish the situation was even more pronounced, 85% of the isolates were oxidase-negative, penicillin- and oxytetracycline-insensitive achromobacters and only 15% were oxidase-positive, penicillin-sensitive and oxytetracycline-sensitive achromobacters. The oxidase-negative achromobacters were not trimethylamine producers and at spoilage

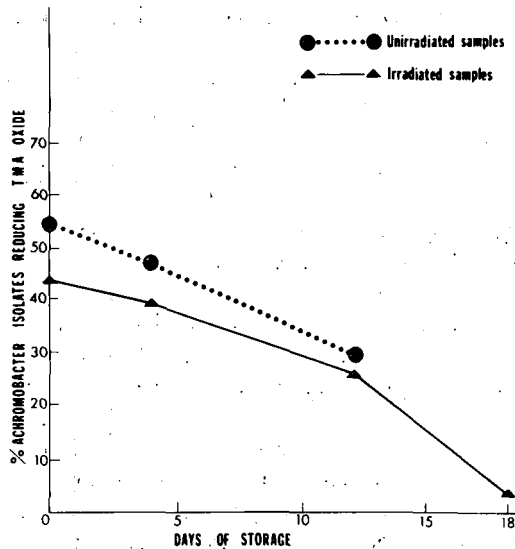


FIG.6. The proportion of Achromobacter isolates producing TMA during storage at 3°C (average results for 2- and 5-day fish).

the 2-day and 5-day irradiated fish had only 2% and 6% trimethylamine producers, respectively.

Pseudomonads were isolated during storage of irradiated fish but apparently were not able to outgrow the achromobacters after irradiation. In 2-day fillets Pseudomonas I and II were of the order of 10^4 per gram after 4 days of storage and still of the same order after 8 days of storage after which they were not further detected. Pseudomonas putrefaciens was not isolated from 2-day irradiated fish. On 5-day fillets Pseudomonas I and II were reduced to around 50 per gram on irradiation and were not again detected. Pseudomonas putrefaciens was isolated only at the time of spoilage when it had reached a level of 10^7 per gram. In 9-day fish Pseudomonas I and II and Pseudomonas putrefaciens formed a small percentage of the isolates immediately after irradiation and at the time of spoilage had increased to between 10^6 and 10^7 organisms per gram.

4. SUMMARY AND CONCLUSIONS

Irradiation at 100 krad brought about an initial reduction in numbers of bacteria on haddock fillets. At spoilage, however, irradiated 2-day and 5-day fish had between 10 and 100 times as many bacteria as their unirradiated counterparts had at spoilage. Slavin et al. (1966 [21]) and Spinelli et al. (1964, 1965 [22-23]) observed the same effect on cod, petrale sole and king crab and it was suggested that this might be due to the post-irradiation flora

being less metabolically active so that greater numbers were needed to produce an equivalent amount of spoilage.

As far as could be told the initial quality of the unirradiated fillets did not affect the kinds of organisms developing during spoilage, Pseudomonas putrefaciens, Pseudomonas groups I and II and Achromobacter predominated in the control fillets of all ages. In irradiated fillets, Achromobacter was the dominant isolate during spoilage of all fillets. Masurovsky et al. (1963 [15]) found that Pseudomonas grew out and formed a considerable proportion of the spoilage flora of haddock after irradiation at doses between 50 and 300 krad. In the present experiment Pseudomonas was not found to form more than a small percentage of the post-irradiation flora, even in the older fillets. However, it would have been quite possible for both Pseudomonas I and II and Pseudomonas putrefaciens to be present in the 2-day and 5-day irradiated fillets at spoilage in numbers sufficient to cause spoilage, without their being isolated at all. Total bacterial numbers were over 10^9 per gram and only 100 isolates represented them, thus an organism had to be present in number of over 10^7 per gram before forming 1% of the isolates.

Only one of the groups of organisms predominating during storage was consistently capable of producing trimethylamine from trimethylamine oxide, Pseudomonas putrefaciens. The proportions of Achromobacters able to reduce trimethylamine oxide declined during storage of both unirradiated and irradiated fish, and members of Pseudomonas I and II with this capability were rarely isolated. Thus it seems that Pseudomonas putrefaciens, a known spoiler, may be, in these experiments at least, the link between spoilage and trimethylamine production. The organism was isolated during spoilage from all treatments, and was known to be present in high numbers at spoilage in all except the 2-day irradiated fish, in which, statistically, it could have been present in numbers of over 10^7 per gram without being isolated.

ACKNOWLEDGEMENT

This study was supported in part by Atomic Energy of Canada Limited.

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PROLONGING THE COMMERCIAL LIFE OF FRESH WHITE (LEAN) FISH: MICROBIOLOGICAL ASPECTS

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Abstract

PROLONGING THE COMMERCIAL LIFE OF FRESH WHITE (LEAN) FISH: MICROBIOLOGICAL ASPECTS.

The author describes a series of experiments on the irradiation of fresh white (lean) fish of the species Merluccius merluccius, in which the dose applied ranged between 50 and 300 krad and the irradiation conditions, i.e. packaging in different materials, temperature and atmosphere, were varied. The microbiological studies were concentrated on the evolution of residual microbial flora as a function of the parameters considered. The results obtained are discussed with a view to evaluating their significance from the commercial and health standpoints. It is concluded that under suitable conditions, with storage temperatures from 0 to 3°C and low initial contamination levels, it is possible to secure a commercially worthwhile prolongation of the storage life of hake fillets amounting to 10 to 12 days, by the application of doses of 50 and 100 krad. Larger doses are required for higher initial contamination indices and also whenever gas-permeable wrapping materials are used. It will be necessary, however, to make further tests to confirm the conclusions, and in so doing to broaden and improve various aspects and areas of the scheduled research.

1. INTRODUCTION

Under Research Contract No. 731/RB awarded by the International Atomic Energy Agency for implementation by the Spanish Nuclear Energy Board, we have carried out five experiments on low-level irradiation of fish of the species Merluccius merluccius. This species has merited special attention on account of the volume in which it is caught and the economic interest which it presents. The work performed covers the first nine months of the contract referred to, which was begun early this year (1969) and which is entitled: Radiation Sensitivity of the Microbial Flora of Fresh White (Lean) Fish.

The aim of our work is twofold: first, to make microbiological, biochemical and organoleptic studies on the development and evolution of the chief groups of micro-organisms in irradiated fish as a function of the radiation dose and conditions, and, second, to lay a basis of technology and health protection on which to develop the processing and marketing of fish irradiated by low doses.

In view of the experience gained by other establishments in the irradiation of fish; first and foremost those in the United States of America, United Kingdom, Canada and Iceland, we are seeking to acquire our own experience,

using parameters that we feel are of technical and commercial importance and applying microbiological techniques that will enable us to assess the quality of the fish at the same time as guaranteeing that it is acceptable to the health authorities.

The results that have been obtained so far are definitely encouraging, but we are aware that there are still many outstanding problems to be solved in various areas. We hope to be able to contribute to the solution of these problems by means of our programs of work and to attain specific objectives of basically practical interest that can pave the way for official authorization of the consumption by the public of fish irradiated on a commercial scale.

2. MATERIAL AND METHODS

The irradiation tests carried out on fillets of whiting and hake included an analysis of microbiological control and study of the common flora of marine origin and flora resulting from contamination on land. All the irradiations were carried out in the "Nayade" irradiation unit belonging to the Isotope Section of the Spanish Nuclear Energy Board (JEN) [1]. Use was also made of equipment and material normally found in all microbiology laboratories.

The work undertaken was divided into a series of experiments. The preliminary tests were followed by other, more specific experiments aimed at a separate study of the effects produced by the parameters used.

2.1. Microbiological techniques employed

The fish fillets were sampled by taking approximately 5 grams of muscle tissue, under aseptic conditions, in such a way that the sample also formed a strip 5 cm² in area; this was done so as to be able to make both bacteria/g and bacteria/cm² counts. The samples were introduced into glass vessels containing peptone water that had been previously autoclaved, so as to form a 10% dilution, which was then macerated using an Ultraturrax blender.

(a) Total mesophilic aerobic and anaerobic microflora

The total microflora count was made by heavy inoculation in Petri dishes containing Plate Count Agar (PCA) made by Difco, using the decimal dilution technique, and by cultivation in 160 × 16 mm tubes with AC Medium (also made by Difco). With the latter culture medium it is possible to count aerobic in addition to anaerobic bacteria with satisfactory approximation, since it contains ascorbic acid as well as the other ingredients. Strict and facultative anaerobic bacteria were determined using PCA and Nutrient Agar (made by Difco), following cultivation in long 420 × 8 mm tubes.

For each dilution the inoculations were carried out in duplicate and triplicate.

Incubation lasted 72 hours at two temperatures: 30°C in the incubator, and room temperature, which varied between 18 and 20°C.

(b) Psychrophilic bacteria

Marin Agar (Difco) as well as PCA were used as culture media for enumerating these bacteria. Incubation at 4-6°C lasted 12 days and at 15°C six days.

(c) Escherichia coli I and coliforms

Escherichia coli I was studied using the MacKenzie test: inoculation in brilliant green broth with 2% bile, and in a Durham fermentation tube with peptone water; incubation at 44°C for 24 hours.

The coliform bacteria were counted by preliminary inoculation of (sodium) desoxycholate agar, followed by incubation at 37°C for 48 hours. To determine the bacteria producing H₂S, 0.5% ferric citrate and 0.2% cystine were added to the peptone water.

The indole test was carried out with Ehrlich-Kovacs's reagent.

(d) Clostridium welchii

Clostridium perfringens, both welchii and the sulphite-reducing types in general, were studied with the use of Iron Sulphite Agar (made by Oxoid Ltd.). Incubation at 44°C for 24 hours.

(e) Pathogenic staphylococci

Coagulase-positive staphylococci were counted using Baird-Parker's medium, the inoculation technique being modified for use with intermediate diluent tubes, as described by Fournaud et al. [2].

(f) Salmonella and other pathogenic enterobacteriaceae

These micro-organisms were studied by various isolation techniques involving previous enrichment with selenite broth containing cystine, and (sodium) tetrathionate broth. In view of the possibility of there being only a small number of Salmonella colonies, we adopted the following procedure to detect them:

First day: to a suspension of the sample in peptone water that had been kept at room temperature for six hours we added an equal volume of selenite broth with cystine, and incubated at 43°C for 18 hours.

Second day: droplets were removed from the suspension described above, using a platinum loop, and streak plates were made with brilliant green agar (AVB) and (sodium) desoxycholate-citrate-lactose agar (DCLS) in Petri dishes.

Third day: tubes containing a Kligler Iron Agar slope culture were inoculated with suspected colonies, but so far the existence of Salmonella has not been established by any of the tests.

(g) Identification of bacterial types and percentage composition of the microflora

For the isolation and differentiation of various bacterial groups we used selective techniques, which in conjunction with microscopic observations made it possible to identify them. In these

TABLE I. FIRST PRELIMINARY EXPERIMENT

Results of microbiological tests 21 days after irradiation: dose - 300 krad

H ₂ S test		Bacteria count							
		Aerobic (bac./g)				Anaerobic (bac./g)			
Control	Irradiated	Incubated at 30°C		Ambient temperature		Incubated at 30°C		Ambient temperature	
Positive up to 10 ⁶ dilution	Negative in all dilutions	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated
		3 × 10 ⁹	3 × 10 ⁵	2 × 10 ⁹	4 × 10 ⁵	10 ⁸	10 ³	10 ⁸	0

TABLE II. SECOND PRELIMINARY EXPERIMENT

Results of microbiological tests: dose - 300 krad

Date	H ₂ S test (bac./g)		Total aerobic microflora (bac./g)	
	Control	Irradiated	Control	Irradiated
1st day	Positive 10 ⁵	Negative	6.3 × 10 ⁵	10 ²
21st day	Positive 10 ⁶	Negative	3.4 × 10 ⁷	1.2 × 10 ⁵

techniques we followed the classification contained in Bergey's Manual of Determinative Bacteriology [3].

2.2. Parameters studied

With the described objectives in mind we studied the total mesophilic, psychrophilic, aerobic and anaerobic microflora, including the genus Clostridium. We similarly determined the optimum radiation dose, the nitrogen in the total volatile bases, the nitrogen in trimethylamine, and the parameters that are described below.

2.2.1. Radiation dose

In the preliminary experiments a dose of 300 krad was applied, and in subsequent tests doses of 50, 100, 150 and 200 krad. The dose rate was 0.41 Mrad/h, except in the final experiment in which a dose rate of 1.134 Mrad/h was applied.

2.2.2. Initial contamination levels

The shipments of fish originated from different sources so that we could evaluate the influence of the initial contamination levels and the time lapsing from the moment of catch on prolongation of the storage life of the irradiated specimens.

2.2.3. Irradiation conditions

A variety of packaging materials and different atmospheres was used. Use was first made of polyethylene bags and then strips of nylon known under the trade name of "Rilsan". The initial experiments were carried out in a normal atmosphere and the last two were made with bags sealed under vacuum.

3. PRELIMINARY EXPERIMENTS

These experiments were performed with fish samples originating from a retail market so as to gain an idea of the true conditions of initial contamination in this grade of marketed fish, as purchased by the housewife. In these tests a single dose of 300 krad was applied, polyethylene being used as the wrapping material. The storage temperature was kept at $+3^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

TABLE III. RESULTS OF THE FIRST EXPERIMENT
Week 0. Storage temperature +3°C

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	5.0 ^a	3.2	2.1	2.0
	anaerobic	2.2	< 2.0	1.8	1.6
	psychrophilic	5.1	> 3.0	> 2.0	2.2
Biochemical tests	H ₂ S	> 2.0	-	-	-
	Indole	-	-	-	-
	NH ₃	-	-	-	-
Coliforms in (sodium) desoxycholate		2.3	-	-	-
MacKenzie test		-	-	-	-
Enterococci		-	-	-	-
Moulds and yeast		> 2.0	< 2.0	< 2.0	1.6
Staphylococci (Baird-Parker)		< 1.0	< 1.0	< 1.0	< 1.0
Salmonella (Kauffmann)		-	-	-	-

Storage temperature +8-10°C

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	5.1	3.3	< 3.0	2.1
	anaerobic	2.5	2.0	1.5	1.2
	psychrophilic	5.0	3.2	< 3.0	< 2.0
Biochemical tests	H ₂ S	3.0	-	-	-
	Indole	-	-	-	-
	NH ₃	-	-	-	-
Coliforms in (sodium) desoxycholate		3.4	-	-	-
MacKenzie test		-	-	-	-
Enterococci		-	-	-	-
Moulds and yeast		> 2.0	< 2.0	< 2.0	1.4
Staphylococci (Baird-Parker)		< 1.0	< 1.0	< 1.0	< 1.0
Salmonella (Kauffmann)		-	-	-	-

^a The results are expressed in log₁₀ of the number of viable micro-organisms per gram.

TABLE IV. RESULTS OF THE FIRST EXPERIMENT
Week 1. Storage temperature +3°C.

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	6.1 ^a	3.3	2.3	2.0
	anaerobic	2.5	2.0	2.0	2.0
	psychrophilic	5.2	3.1	2.2	2.1
Biochemical tests	H ₂ S	> 3.0	-	-	-
	Indole	-	-	-	-
	NH ₃	-	-	-	-
Coliforms in (sodium) desoxycholate		4.3	-	-	-
MacKenzie test		-	-	-	-
Enterococci		1.0	-	-	-
Mould and yeast		> 3.0	< 2.0	1.0	1.0
Staphylococci (Baird-Parker)		< 1.0	< 1.0	< 1.0	< 1.0
<u>Salmonella</u> (Kauffmann)		-	-	-	-

Storage temperature +8-10°C.

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	7.2	5.1	4.4	3.2
	anaerobic	4.6	3.1	2.3	2.0
	psychrophilic	6.5	4.6	4.0	2.8
Biochemical tests	H ₂ S	5.0	1.0	-	-
	Indole	3.0	-	-	-
	NH ₃	2.0	-	-	-
Coliforms in (sodium) desoxycholate		4.3	2.2	1.0	1.0
MacKenzie test		2.0	-	-	-
Enterococci		1.0	-	-	-
Moulds and yeast		3.1	2.02	< 2.0	< 2.0
Staphylococci (Baird-Parker)		< 1.0	< 1.0	< 1.0	< 1.0
<u>Salmonella</u> (Kauffmann)		-	-	-	-

^a The results are expressed in log₁₀ of the number of viable micro-organisms per gram.

TABLE V. RESULTS OF THE FIRST EXPERIMENT
Week 2. Storage temperature +3°C

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	8.1 ^a	5.0	3.8	3.0
	anaerobic	5.6	3.4	2.1	2.2
	psychrophilic	7.4	4.5	3.3	3.1
Biochemical tests	H ₂ S	eliminated	-	-	-
	Indole	"	-	-	-
	NH ₃	"	-	-	-
Coliforms in (sodium) desoxycholate		"	2.0	2.0	2.0
MacKenzie test		"	-	-	-
Enterococci		"	-	-	-
Moulds and yeast		"	3.0	3.0	2.1
Staphylococci (Baird-Parker)		"	< 1.0	< 1.0	< 1.0
<u>Salmonella</u> (Kauffmann)		"	-	-	-

Storage temperature +8-10°C

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	eliminated	7.2	5.7	4.5
	anaerobic	"	4.0	3.4	2.5
	psychrophilic	"	5.8	4.2	4.0
Biochemical tests	H ₂ S	"	3.0	-	-
	Indole	"	-	-	-
	NH ₃	"	-	-	-
Coliforms in (sodium) desoxycholate		"	3.3	< 2.0	< 2.0
MacKenzie test		"	-	-	-
Enterococci		"	-	-	-
Moulds and yeast		"	3.4	2.5	< 2.0
Staphylococci (Baird-Parker)		"	< 1.0	< 1.0	< 1.0
<u>Salmonella</u> (Kauffmann)		"	-	-	-

^a The results are expressed in log₁₀ of the number of viable micro-organisms per gram.

TABLE VI. RESULTS OF THE FIRST EXPERIMENT
Week 3. Storage temperature +3°C

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	eliminated	6.0 ^a	4.0	3.6
	anaerobic	"	3.8	2.3	2.5
	psychrophilic	"	5.7	3.2	3.3
Biochemical tests	H ₂ S	"	3.0	-	-
	Indole	"	-	-	-
	NH ₃	"	-	-	-
Coliforms in (sodium) desoxycholate		"	3.1	2.2	-
MacKenzie test		"	-	-	-
Enterococci		"	2.0	-	-
Moulds and yeast		"	3.5	2.2	< 2.0
Staphylococci (Baird-Parker)		"	-	-	-
<u>Salmonella</u> (Kauffmann)		"	-	-	-

Storage temperature +8-10°C

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	eliminated	6.0	4.4	4.1
	anaerobic	"	4.2	3.4	2.2
	psychrophilic	"	5.8	4.5	4.2
Biochemical tests	H ₂ S	"	eliminated	1.0	1.0
	Indole	"	"	-	-
	NH ₃	"	"	-	-
Coliforms in (sodium) desoxycholate		"	"	2.0	2.0
MacKenzie test		"	"	-	-
Enterococci		"	"	-	-
Moulds and yeast		"	"	3.8	4.0
Staphylococci (Baird-Parker)		"	"	-	-
<u>Salmonella</u> (Kauffmann)		"	"	-	-

^a The results are expressed in log₁₀ of the number of viable micro-organisms per gram.

TABLE VII. RESULTS OF THE FIRST EXPERIMENT
Week 4. Storage temperature +3 °C

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	eliminated	7.3 ^a	6.5	4.5
	anaerobic	"	3.8	3.5	3.0
	psychrophilic	"	7.0	6.1	4.2
Biochemical tests	H ₂ S	"	3.0	1.0	-
	Indole	"	-	-	-
	NH ₃	"	-	-	-
Coliforms in (sodium) desoxycholate		"	3.0	3.0	2.0
MacKenzie test		"	-	-	-
Enterococci		"	1.0	-	-
Moulds and yeast		"	4.0	3.7	2.5
Staphylococci (Baird-Parker)		"	-	-	-
<u>Salmonella</u> (Kauffmann)		"	-	-	-

Storage temperature +8-10 °C

Microbiological examination		Control	100 krad	200 krad	300 krad
Total microflora count	aerobic and anaerobic	eliminated	eliminated	eliminated	7.5
	anaerobic	"	"	"	6.1
	psychrophilic	"	"	"	7.2
Biochemical tests	H ₂ S	"	"	"	2.0
	Indole	"	"	"	-
	NH ₃	"	"	"	-
Coliforms in (sodium) desoxycholate		"	"	"	2.0
MacKenzie test		"	"	"	-
Enterococci		"	"	"	1.0
Moulds and yeast		"	"	"	4.6
Staphylococci (Baird-Parker)		"	"	"	-
<u>Salmonella</u> (Kauffmann)		"	"	"	-

^a The results are expressed in log₁₀ of the number of viable micro-organisms per gram.

The fish was packaged in filleted form in commercial polyethylene bags, each of which contained approximately 100 g; the outer dimensions of the bags were $7 \times 7 \times 3.2$ cm. 25% of the samples were left unirradiated to serve as a control. The results of the microbiological examinations are shown in Tables I and II.

4. FIRST EXPERIMENT: Irradiation and preservation at two temperatures

To compare the effect of temperature variation on the development and evolution of microflora originating from the irradiated fish, we procured 10 kg of hake from the Central Fish Market for which data were available on the area, date and mode of catch; the fish was prepared in similar fashion to the previous experiments, but then subjected to various radiation doses as follows:

- 25% of the fillets were irradiated at 100 krad;
- 25% of the fillets were irradiated at 200 krad;
- 25% of the fillets were irradiated at 300 krad;
- 25% of the fillets were not irradiated at all (control).

The 50 packets containing 100 g of prepared fish were divided into two equal batches which were stored separately at $\pm 3^\circ\text{C}$ and at $8-10^\circ\text{C}$. The results are shown in detail in Tables III to VII.

TABLE VIII. RESULTS OF THE SECOND EXPERIMENT

Samples sealed in a normal atmosphere. Temperature $+2^\circ\text{C} \pm 1^\circ\text{C}$
Week 0

Microbiological examination		Control	100 krad	150 krad	200 krad
Total microflora count	aerobic and anaerobic	5.3 ^a	3.8	3.8	2.6
	anaerobic	2.8	1.5	1.6	1.0
	psychrophilic	5.1	3.2	3.1	2.2
Biochemical tests	H ₂ S	3.0	-	-	-
	Indole	-	-	-	-
	NH ₃	-	-	-	-
Coliforms in (sodium) desoxycholate		2.4	-	-	-
MacKenzie test		-	-	-	-
Moulds and yeasts		< 1.1	< 1.0	< 1.0	< 1.0
Sulphite-reducing <u>Clostridium</u>		-	-	-	-

^a The results are expressed in $\log_{10}$ of the number of viable micro-organisms per gram.

TABLE IX. RESULTS OF THE SECOND EXPERIMENT
Week 1. Samples sealed in a normal atmosphere

Microbiological examination		Control	100 krad	150 krad	200 krad
Total microflora count	aerobic and anaerobic	7.6 ^a	4.2	3.6	3.0
	anaerobic	3.5	2.7	2.1	1.8
	psychrophilic	7.1	4.3	2.8	2.6
Biochemical tests	H ₂ S	3.3	-	-	-
	Indole	-	-	-	-
	NH ₃	-	-	-	-
Coliforms in (sodium) desoxycholate		5.1	1.5	1.0	1.0
MacKenzie test		-	-	-	-
Moulds and yeasts		2.2	< 2.0	1.0	< 2.0
Sulphite-reducing <u>Clostridium</u>		-	-	-	-

Samples sealed under vacuum

Microbiological examination		Control	100 krad	150 krad	200 krad
Total microflora count	aerobic and anaerobic	7.9	4.1	4.0	3.2
	anaerobic	4.3	2.1	2.0	2.0
	psychrophilic	6.9	4.2	4.1	3.0
Biochemical tests	H ₂ S	3.0	-	-	-
	Indole	-	-	-	-
	NH ₃	-	-	-	-
Coliforms in (sodium) desoxycholate		5.1	-	-	-
MacKenzie test		-	-	-	-
Moulds and yeasts		2.3	1.6	1.2	1.4
Sulphite-reducing <u>Clostridium</u>		-	-	-	-

^a The results are expressed in log₁₀ of the number of viable micro-organisms per gram.

TABLE X. RESULTS OF THE SECOND EXPERIMENT
Week 2. Samples sealed in a normal atmosphere

Microbiological examination		Control	100 krad	150 krad	200 krad
Total microflora count	aerobic and anaerobic	9.4 ^a	4.5	3.2	3.3
	anaerobic	4.8	2.6	1.8	1.2
	psychrophilic	8.8	4.1	3.4	2.8
Biochemical tests	H ₂ S	eliminated	2.0	-	-
	Indole	"	-	-	-
	NH ₃	"	-	-	-
Coliforms in (sodium) desoxycholate		"	3.0	-	-
MacKenzie test		"	-	-	-
Moulds and yeasts		"	2.3	2.4	2.0
Sulphite-reducing <u>Clostridium</u>		"	-	-	-

Samples sealed under vacuum

Microbiological examination		Control	100 krad	150 krad	200 krad
Total microflora count	aerobic and anaerobic	eliminated	4.9	4.4	3.1
	anaerobic	"	3.1	3.3	2.1
	psychrophilic	"	4.5	4.6	2.8
Biochemical tests	H ₂ S	"	1.0	-	-
	Indole	"	-	-	-
	NH ₃	"	-	-	-
Coliforms in (sodium) desoxycholate		"	3.1	-	-
MacKenzie test		"	-	-	-
Moulds and yeasts		"	2.2	1.6	1.0
Sulphite-reducing <u>Clostridium</u>		"	-	-	-

^a The results are expressed in log₁₀ of the number of viable micro-organisms per gram.

TABLE XI. RESULTS OF THE SECOND EXPERIMENT
Week 3. Samples sealed in a normal atmosphere

Microbiological examination		Control	100 krad	150 krad	200 krad
Total microflora count	aerobic and anaerobic	eliminated	5.1 ^a	4.7	4.4
	anaerobic	"	3.0	2.2	2.0
	psychrophilic	"	5.2	4.1	3.2
Biochemical tests	H ₂ S	"	3.0	-	-
	Indole	"	-	-	-
	NH ₃	"	-	-	-
Coliforms in (sodium) desoxycholate		"	3.3	-	-
MacKenzie test		"	-	-	-
Moulds and yeasts		"	3.1	3.0	3.0
Sulphite-reducing <u>Clostridium</u>		"	-	-	-

Samples sealed under vacuum

Microbiological examination		Control	100 krad	150 krad	200 krad
Total microflora count	aerobic and anaerobic	eliminated	7.1	6.2	4.4
	anaerobic	"	3.5	3.3	3.9
	psychrophilic	"	6.7	5.8	4.2
Biochemical tests	H ₂ S	"	2.0	2.0	1.0
	Indole	"	-	-	-
	NH ₃	"	-	-	-
Coliforms in (sodium) desoxycholate		"	3.1	-	-
MacKenzie test		"	3.4	2.6	3.1
Moulds and yeasts		"	-	-	-
Sulphite-reducing <u>Clostridium</u>		"	-	-	-

^a The results are expressed in log₁₀ of the number of viable micro-organisms per gram.

TABLE XII. RESULTS OF SECOND EXPERIMENT
Week 4. Samples sealed in a normal atmosphere

Microbiological examination		Control	100 krad	150 krad	200 krad
Total microflora count	aerobic and anaerobic	eliminated	7.2 ^a	5.3	5.2
	anaerobic	"	4.1	3.4	3.6
	psychrophilic	"	6.1	5.0	4.6
Biochemical tests	H ₂ S	"	3.0	1.0	-
	Indole	"	-	-	-
	NH ₃	"	-	-	-
Coliforms in (sodium) desoxycholate		"	3.5	1.0	-
MacKenzie test		"	1.0	-	-
Moulds and yeasts		"	2.8	2.5	2.6
Sulphite-reducing <u>Clostridium</u>		"	-	-	-

^a The results are expressed in log₁₀ of the number of viable micro-organisms per gram.

5. SECOND EXPERIMENT: Application of different radiation doses to polyethylene-wrapped fish samples

Upon receipt by the laboratory of 22 kg of hake in seven pieces, each weighing roughly 3 kg and originating from the same source as before, we filleted the fish and packaged it in commercial polyethylene bags. In this series of tests the doses applied were 100, 150 and 200 krad. Control analysis was performed on a weekly basis until the fourth week so as to study the physico-chemical and microbiological evolution of the samples, as compared to other control samples kept under the same conditions. The storage temperature was $+2^{\circ} \pm 1^{\circ}\text{C}$. Tables VIII to XII indicate the results obtained.

6. THIRD EXPERIMENT: Irradiation tests involving the delivery of 100 krad to fish samples sealed under vacuum and in a normal atmosphere

Twenty-one kilograms of hake was procured from the same supplier. After filleting it was packaged at the laboratory in four batches in the following manner:

- 25% of the fillets, irradiated, vacuum-sealed ;
- 25% of the fillets, non-irradiated, vacuum-sealed ;

TABLE XIII. THIRD EXPERIMENT

Irradiation tests involving the delivery of 100 krad to fish samples packaged and sealed under vacuum and in a normal atmosphere

Dose and conditions (krad)	Storage time (days)	Total mesophilic aerobic microflora (bac./g)	Strict facultative anaerobic bacteria (bac./g)	Psychrophilic microflora (bac./g)	Biochemical tests (bac./g)			Coliforms (bac./g)	Sulphite-reducing <i>Clostridium</i> (bac./g)	Moulds and yeasts (bac./g)
					H ₂ S	Indol	NH ₃			
100 Vacuum	0	10 ³	10 ²	10 ³	-	-	-	< 10	-	< 10
	4	5 × 10 ⁴	10 ³	10 ⁴	-	-	-	< 10	-	10 ²
	7	4 × 10 ⁶	10 ⁴	10 ⁵	10 ²	-	-	10 ²	-	10 ²
	11	3 × 10 ⁵	10 ⁴	10 ⁵	10 ²	-	-	10 ²	-	10 ³
	15	5 × 10 ⁶	10 ⁵	10 ⁶	10 ⁴	-	-	10 ²	-	10 ⁴
	18	9 × 10 ⁵	10 ⁵	10 ⁵	10 ⁵	-	-	10 ³	-	10 ³
Control: vacuum	0	5 × 10 ⁴	10 ²	10 ⁴	10 ³	-	-	10 ³	-	10 ²
	4	6 × 10 ⁶	10 ²	10 ⁶	10 ⁵	-	-	5 × 10 ³	-	10 ²
	7	5 × 10 ⁷	10 ²	destroyed	destroyed	destroyed	destroyed	destroyed	destroyed	destroyed
	11	destroyed	10 ³	"	"	"	"	"	"	"
	15	"	destroyed	"	"	"	"	"	"	"
	18	"	"	"	"	"	"	"	"	"
100 Normal atmosphere	0	10 ³	10 ²	10 ³	-	-	-	-	-	< 10
	4	10 ⁴	10 ²	10 ⁴	10 ²	-	-	< 10	-	< 10
	7	5 × 10 ⁴	10 ²	10 ⁴	10 ²	-	-	< 10	-	10 ²
	11	3 × 10 ⁵	10 ³	10 ⁵	10 ²	-	-	6 × 10 ²	-	10 ²
	15	5 × 10 ⁶	10 ³	10 ⁶	10 ³	-	-	10 ²	-	10 ²
	18	3 × 10 ⁶	10 ³	10 ⁶	10 ³	-	-	10 ²	-	10 ³
Control: normal atmosphere	0	4 × 10 ⁴	10 ²	10 ⁴	10 ³	-	-	3 × 10 ³	-	< 10
	4	6 × 10 ⁷	10 ³	10 ⁷	10 ⁶	-	-	8 × 10 ⁴	-	10 ²
	7	destroyed	destroyed	destroyed	destroyed	-	-	destroyed	-	-
	11	"	"	"	"	-	-	-	-	-
	15	"	"	"	"	-	-	-	-	-
	18	"	"	"	"	-	-	-	-	-

- 25% of the fillets, irradiated, sealed in a normal atmosphere;
- 25% of the fillets, non-irradiated, sealed in a normal atmosphere.

All the samples were stored at a temperature of $+2^{\circ} \pm 0.5^{\circ}\text{C}$. The results are shown in Table XIII.

7. FOURTH EXPERIMENT: Microbiological studies of white fish irradiated at low doses and packaged in different atmospheres

In order to carry out this experiment we made arrangements in the fishing port of Vigo to have the fish shipped to the laboratory under the best possible conditions, with a record of its history from the time it was caught. Under these conditions we received the first shipment containing three hake, each weighing roughly 8 kg. After preparing 100 packages of 100 g, we irradiated them with doses of 50, 100 and 150 krad, retaining 25% of the packages as the control. Half of the samples were sealed under vacuum and the other half in a normal atmosphere. We thereby obtained eight batches of fish samples divided up in the following manner:

- 12.5% irradiated with 50 krad and vacuum-sealed;
- 12.5% irradiated with 100 krad and vacuum-sealed;
- 12.5% irradiated with 150 krad and vacuum-sealed;
- 12.5% non-irradiated and vacuum-sealed (control);
- 12.5% irradiated with 50 krad and sealed in a normal atmosphere;
- 12.5% irradiated with 100 krad and sealed in a normal atmosphere;
- 12.5% irradiated with 150 krad and sealed in a normal atmosphere;
- 12.5% non-irradiated and sealed in a normal atmosphere (control).

The quality of the fish and the short time elapsing between when it was caught and its arrival at the laboratory (72 hours) were checked during the analyses of the initial contamination indices; the result was a marine and terrestrial bacterial level of less than 10^3 bac./g.

The results of fortnightly tests are given in Table XIV.

8. DISCUSSION OF RESULTS

In the preliminary tests that were carried out on whiting samples with an initial contamination of the order of 10^5 bac./g, the application of a dose of 300 krad held up decomposition of the fish for 21 days. The differences in quality between the control and irradiated samples became clear, starting from the first week. The former had to be destroyed on the seventh day, while the irradiated samples remained fit for consumption, from the microbiological and biochemical point of view, for up to three weeks.

The H_2S test was positive in the case of the control samples down to a dilution of 10^3 from 48 hours following reception of the fish, whereas in the irradiated samples the result was negative up to 21 days, including for a dilution of 1/10.

TABLE XIV. FOURTH EXPERIMENT

Results of microbiological studies on white fish irradiated at low doses and packaged in different atmospheres

Dose and conditions (krad)	Storage time (days)	Total mesophilic aerobic microflora (bac./g)	Coliforms (bac./g)	Biochemical tests (bac./g)			Moulds and yeasts (bac./g)
				H ₂ S	Indole	NH ₃	
50 Vacuum	0	5×10^2	negative	-	-	-	< 10
	5	3×10^3	"	10^2	-	-	10^2
	11	4×10^4	"	10^3	-	-	10^3
	14	3×10^5	$> 10^2$	10^2	-	-	10^3
	18	5×10^5	$> 10^2$	10^2	-	-	10^3
	21	6×10^5	$> 10^2$	10^3	-	-	10^3
100 Vacuum	0	10^2	negative	-	-	-	< 10
	5	5×10^3	"	-	-	-	< 10
	11	2×10^3	"	10	-	-	10^2
	14	3×10^4	"	10^3	-	-	10^2
	18	9×10^5	"	10^2	-	-	10^3
	21	7×10^6	"	10^3	-	-	10^2
150 Vacuum	0	10^2	negative	-	-	-	< 10
	5	2×10^3	"	-	-	-	< 10
	11	10^3	"	-	-	-	< 10
	14	10^4	"	10	-	-	10^2
	18	4×10^5	"	10	-	-	10^2
	21	3×10^5	"	10	-	-	10^2
Control vacuum	0	3×10^3	< 10	10^3	-	-	< 10
	5	8×10^4	$> 10^3$	10^4	-	-	10^2
	11	5×10^7	$> 10^3$	10^4	10	destroyed	10^2
	14	destroyed	destroyed	destroyed	destroyed	"	destroyed
	18	"	"	"	"	"	"
	21	"	"	"	"	"	"
50 Normal atmosphere	0	2×10^3	negative	-	-	-	< 10
	5	9×10^4	"	-	-	-	< 10
	11	7×10^4	"	10^2	-	-	10^2
	14	3×10^6	$> 10^2$	10^2	-	-	10^2
	18	8×10^5	$> 10^2$	10^4	-	-	10^3
	21	5×10^6	$> 10^3$	10^3	-	-	10^5
100 Normal atmosphere	0	3×10^3	negative	10	-	-	< 10
	5	7×10^4	"	10	-	-	< 10
	11	3×10^4	"	10	-	-	< 10
	14	10^5	"	10	-	-	10^2
	18	6×10^5	"	10	-	-	10^2
	21	2×10^6	$> 10^2$	10^2	-	-	10^3

Dose and conditions (krad)	Storage time (days)	Total mesophilic aerobic microflora (bac./g)	Coliforms (bac./g)	Biochemical tests (bac./g)			Moulds and yeasts (bac./g)
				H ₂ S	Indole	NH ₃	
150 Normal atmosphere	0	10 ²	negative	-	-	-	< 10
	5	2 × 10 ³	"	-	-	-	10 ²
	11	5 × 10 ³	"	-	-	-	< 10
	14	8 × 10 ⁵	"	10	-	-	10 ²
	18	9 × 10 ⁴	"	10 ²	-	-	10 ²
	21	7 × 10 ⁵	"	10	-	-	10 ⁴
Control: normal atmosphere	0	5 × 10 ³	> 10 ³	10 ³	-	-	< 10
	5	8 × 10 ⁴	> 10 ³	10 ³	-	-	< 10
	11	7 × 10 ⁶	> 10 ³	10 ³	-	-	10 ²
	14	4 × 10 ⁷	> 10 ⁴	10 ³	-	-	10 ²
	18	9 × 10 ⁷	> 10 ³	10 ⁴	-	-	10 ⁴
	21	destroyed	destroyed	destroyed	-	-	10 ⁴

The total aerobic counts for mesophilic and psychrophilic microflora, just as those for the anaerobic bacteria made immediately after irradiation, showed that the initial contamination mentioned of 10⁵ bac./g was reduced to 10² bac./g.

The results of the four subsequent experiments demonstrated that in order to prolong the storage life for the minimum amount of time that can be considered commercially worthwhile, it is sufficient to apply lower doses than those used for the preliminary tests.

At doses of 300 krad we perceived changes, even though they were slight, in the organoleptic properties, whereas at doses of the order of 100 krad no appreciable variation was detected in the natural characteristics of the fresh fish, as against the untreated samples, and the storage period was prolonged to a sufficient degree to be of commercial interest.

Study of Gram-negative bacteria, particularly Pseudomonas and the coli-form group, by plate counts with nutritive agar and sodium desoxycholate agar, showed the greatest radiation sensitivity among these bacteria as compared to other groups, and doses of 50 and 100 krad proved sufficient to destroy the majority of them. On the other hand, enterococci and other Gram-positive bacteria showed greater radiation resistance, though with certain exceptions.

The optimum dose at the present time considered necessary to prolong the commercial life of the fish to the minimum worthwhile extent has been shown by the experiments performed to depend very considerably on the initial contamination level. Hence, in the last experiment carried out with hake procured directly from the port of Vigo, 50- and 100-krad doses proved adequate in prolonging the storage period by 12-18 days, provided that the fish fillets are kept refrigerated at 2-3°C and are wrapped in plastic materials that are not gas-permeable.

The results obtained from the various experiments relating to microbiological analysis aimed at detecting the pathogenic bacteria that are possible causes of food poisoning did not enable us to confirm the presence of bacteria of the genera Clostridium, Staphylococcus and Salmonella, although the culture media employed did reveal the presence of certain enterobacteriaceae. We therefore consider that when dealing with products that have been handled it is possible that there is contamination by toxic bacteria of terrestrial origin that require systematic study.

As regards the identification of the bacteria and approximate calculation of the percentage composition of the residual flora, we feel that the data obtained are of great interest and indicate the influence of the various parameters tried out, chiefly the wrapping materials and different atmospheres. The number of colonies obtained by plating in various culture media is such that we see notable differences in the composition of the flora, and therefore in the different decomposition mechanisms in the fish, to which we have to attribute the existence of bacterial groups responsible for particular odours when the period by which the commercial life is prolonged has lapsed.

9. GENERAL CONCLUSIONS

From all that has gone before we can arrive at the following conclusions:

1. When radiation doses between 50 and 300 krad are applied to fillets of hake and whiting, they reduce the microbial content by an inactivation factor ranging from 10^2 to 10^3 .
2. Bacteria of the genus Pseudomonas and of the coliform group are approximately 90% destroyed by doses of the order of 100 krad.
3. Successive experiments have proved that in the case of the same dose, prolongation of the commercial life of white fish fillets does not depend only on the initial microbial flora, but also on the properties of the plastic material used for packaging, first and foremost how impermeable it is to gases, and on the type of atmosphere under which the fish is packed. Among the materials tried out, the type of nylon known on the market as Rilsan has so far proved the most effective.
4. From the commercial point of view it may be considered that the doses to be recommended (within the experimental conditions used) are 50 and 100 krad. But in spite of having been able to bring about an improvement in the conditions under which the fish is obtained, we feel it necessary to broaden the scale of the scheduled experiments and carry them out in greater detail.

ACKNOWLEDGEMENTS

This work was made possible through the co-operation, assistance and guidance of Mr. M. del Val Cob, Chief of the Isotope Section of JEN, and

Mr. N. Ortin Suné, Chief of the Applications Group of the same organization, to whom the author is extremely grateful.

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REPORT ON A SURVEY PROJECT IN ICELAND ON THE USE OF RADIATION PASTEURIZATION OF VARIOUS SEAFOODS

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Abstract

REPORT ON A SURVEY PROJECT IN ICELAND ON THE USE OF RADIATION PASTEURIZATION OF VARIOUS SEAFOODS.

A survey project on the irradiation preservation of seafoods, sponsored by the Government of Iceland, the International Atomic Energy Agency and the United States Atomic Energy Commission has been carried out at the Icelandic Fisheries Laboratories. A summary of results obtained on Norway lobster tails, deep-sea shrimp and cod fish is given in this paper.

1. INTRODUCTION

In January 1968 an agreement was made by representatives of the Governments of Iceland, the United States Atomic Energy Commission and the International Atomic Energy Agency to undertake a survey project on the use of ionizing radiation for the preservation of seafoods.

An on-shipboard irradiator of 35 000 Ci ^{60}Co was loaned to the Icelandic Fisheries Laboratories by the USAEC.

The project was started at the Icelandic Fisheries Laboratories in July 1968. The main objective of this survey was to investigate whether or not radiation preservation by irradiation is practical under the conditions existing in the fishing industry and trade in Iceland.

The domestic fish market is very limited, so therefore most of the fresh catch is either salted, dried or frozen and then exported. However, a fair quantity of iced fish is shipped to Europe and sold in the fresh state. Iced seafoods are perishable and have a very limited storage life. The extension of storage life by a preservation method, such as radurization was, therefore, highly desirable and of potentially great importance.

The results which follow, of the experiments carried out during this survey, are based on three progress reports previously submitted to the International Atomic Energy Agency.

2. MATERIALS AND METHODS

2.1. Raw material

The major part of the investigation was concentrated on one species of fish, cod (Gadus callarias) and two crustacean species, Norway lobster

(Nephros norvegicus) and deep-sea shrimp (Pandalus borealis). The raw material was obtained mostly from commercial fish-processing plants and, in some instances, from fishing vessels at the time of landing. The quality of the raw material used in these experiments differed somewhat, which undoubtedly affected the overall results of the storage studies. Because of the complexity of the processing data sought in these studies, separate trials had to be used and the results pooled.

2.2. Packaging material

The Norway lobster tails were packed in metal cans 400×212 (0.5 kg or 500 cm³). The deep-sea shrimp, cod fillets and whole cod were packed in polyethylene plastic bags of 0.07-mm thickness. The bags were either heat-sealed without vacuum or tied.

2.3. Storage temperatures

Samples were stored at either 0-1°C and 5-6°C or at 0-1°C only.

2.4. Blanching

About 1.5 kg of raw material (lobster tails or shrimp) were placed in a wide mesh wire-basket and dipped into 5 litres of boiling water. The temperature of the boiling water was thereby lowered to 85-90°C. After a pre-determined time interval, the basket was removed from the hot water and cooled in cold tapwater for the same length of time. The contents of the basket were then drained and packed.

2.5. Irradiation procedure and dosimetry

Dosimetry was performed prior to each group of experiments. Vials of Fe/Cu solution were placed in the centre of each can [1] in the case of lobster tails and the empty space between the cans was filled with rice. In the study of shrimp and cod fillets the vials were placed between the bags. When the irradiator container was not filled with bags, they were placed in the centre position of the container and the empty space filled with water bags. The maximum/minimum dose ratio and the average dose rate for each product was determined. The difference in dose rate between the centre position receiving the highest dose to that receiving the lowest (the lower corner of the carrier) amounted to a 25% variation. The doses used in the lobster-tail experiments were 1×10^5 , 2×10^5 and 3×10^5 rads. The doses used in the deep-sea shrimp experiments were 0.6×10^5 , 1×10^5 and 2×10^5 rads. The doses used in the experiments on cod fillets and whole cod were 1.5×10^5 , 2×10^5 and 0.5×10^5 rads.

2.6. Sensory evaluation for storage life determination

The sensory evaluation used for the lobster tails was based on a five-points scale taste test (T-test). The tails were boiled in a minimum amount of water for seven minutes and the odour and flavour were scored. The sensory evaluation of the shrimp was done in a similar fashion, except that the shrimp samples were steamed in closed containers, instead of being boiled.

The sensory evaluation test used in this study of cod is based on the odour of the raw (uncooked) products (O-test) and the panelists were requested to record their estimation of age (in days) of the test samples, the emphasis being placed in their decision as to whether the samples were edible or not. This technique was developed at the Technological Laboratory, Bureau of Commercial Fisheries, Gloucester, Mass., USA [2].

2.7. Analytical methods for storage-life determination

2.7.1. Volatile acid number (VAN) determination

The method used was that of the AOAC [3] with minor modifications.

2.7.2. Trimethylamine (TMA) determination

The method used was that of Dyer [4], with minor adaptations.

2.7.3. Total aerobic bacterial counts (TBC)

Samples used for bacterial counts were taken by aseptic sampling. The samples were ground in a meat grinder and aliquots weighed into physiological saline dilution blanks. Serial decimal dilutions were made and total counts made by the pour plate agar method, using an agar medium containing: trypticase, 15 g; phytone 5 g; yeast extract, 5 g; NaCl, 5 g; dextrose, 2.5 g; agar, 15 g; distilled water, 1000 ml.

In addition to total bacterial counts made on all storage-test samples, the bactericidal efficacy of the various pre-treatments and the dose levels of irradiation were also determined.

The incubation temperature was at 22°C for 72 hours and counting was done by the aid of a Quebec colony counter.

3. RESULTS

3.1. Results on the study of radiation pasteurization of Norway lobster tails

Much preliminary work was done to solve the problem of black colour formation (melanosis) in lobster tails, as it soon became obvious that the

black colour of the irradiated lobster tails was more distinct than that of the unirradiated products. Blanching the lobster tails prior to irradiation solved this problem.

In this study the bactericidal efficacy of radurization and of heat treatment (blanching) was determined either singly or in combination. The summary of results of the storage tests of 0-1°C and 5-6°C showing the effect of various treatments on the total bacterial count (TBC), sensory evaluation (T-test) and volatile acid number (VAN) is shown in Table I.

TABLE I. STORAGE LIFE OF LOBSTER TAILS AFTER VARIOUS TREATMENTS

Temperature of storage	Treatment	Dose (rads)	TBC $\leq 1 \times 10^6$ after days ^a	T-test ≤ 2 after days ^a	VAN, at end of experiment
0-1°C	Without blanching	0	1- 6	5- 6	40
"	Without blanching	1×10^5	11-13	12-18	17
"	Without blanching	2×10^5	14	18	12
"	Without blanching	3×10^5	24-25	20	10
"	Blanched 1 min	0	6- 8	12-13	79
"	Blanched 1 min	1×10^5	15	25	16
"	Blanched 1 min	2×10^5	18	29	14
"	Blanched 1 min	3×10^5	29	30	11
"	Blanched 2 min	0	9-13	20-22	12
"	Blanched 2 min	1×10^5	19-26	39	18
"	Blanched 2 min	2×10^5	23	40	8
"	Blanched 2 min	3×10^5	42	45	11
5-6°C	Without blanching	0	1	2	136
"	Without blanching	1×10^5	4	4	20
"	Without blanching	2×10^5	6	6	12
"	Without blanching	3×10^5	9	9	22
"	Blanched 1 min	0	3- 5	5	60
"	Blanched 1 min	1×10^5	8	13	17
"	Blanched 1 min	2×10^5	10	15	12
"	Blanched 1 min	3×10^5	10	17	14
"	Blanched 2 min	0	5- 7	7	9
"	Blanched 2 min	1×10^5	8-15	13	12
"	Blanched 2 min	2×10^5	14	18	11
"	Blanched 2 min	3×10^5	19	21	7

^a Results given as a range signify values obtained in more than one experiment.

From the experimental data on the storage studies, the following may be concluded: the results obtained by the storage-test criteria (T-test, TBC and VAN) indicate that the sensory evaluation (five-point T-test) and the total bacterial count (TBC) at 22°C are in a fair agreement and can be used for determining the product storage life of untreated and only irradiated lobster tails. The TBC is, however, of limited value for assessing the storage life of blanched (for two minutes) only, or blanched and irradiated lobster tails. Volatile acid number (VAN) determinations on the irradiated product (2×10^5 rads) and on the blanched and irradiated product gave unreliable results; the VAN test has, however, its value for the untreated product and also possibly for the product receiving irradiation doses lower than 2×10^5 rads.

The storage life of the product at 0-1°C was twice as long as that at 5-6°C.

The storage life at 0-1°C of lobster tails can be categorized as follows: untreated lobster tails are generally unacceptable after five to six days; irradiated samples had a storage life up to three weeks; samples blanched for two minutes, had a storage life up to four weeks. The longest storage life was obtained when the lobster tails were blanched for two minutes and then irradiated, which resulted in a storage life of about five to six weeks. This was a considerable increase in storage life over both irradiation only and blanching for two minutes only. If judged by appearance alone, the storage life of the samples which were only irradiated would be much less, as all dose levels of irradiation did not prevent melanosis and the highest dose seemed to increase it.

By blanching the product for two minutes, melanosis was inhibited; the blanching treatment also reduces the bacterial load in the raw material considerably. Blanching causes some changes in the physical appearance of the lobster meat at the cut surface.

In conclusion, the most promising results found in this study were those that were obtained when the lobster tails were blanched for two minutes and then irradiated, preferably at a dose level of 2.3×10^5 rads.

The storage life of such a product was found to last up to six weeks at the recommended storage temperature of 0-1°C. The borderline of acceptability for the total bacterial count (TBC) was set at 1×10^6 bacteria per gram, which appears to be too low when compared to the taste acceptability.

3.2. Results on the study of radiation pasteurization of Icelandic deep-sea shrimp

Table II gives the outline of experimental procedures on irradiation of shrimp. The benefits found by blanching the Norway lobster tails were adopted in this study. Storage-life determination was done at one temperature only, i.e. all samples were stored at 0-1°C.

In this study, the number of surviving bacteria after various experimental treatments prior to storage was determined. The bactericidal efficacy of

TABLE II. OUTLINE OF EXPERIMENTAL PROCEDURES ON IRRADIATION OF SHRIMP

<u>Group 1</u>	<u>Group 2</u>	<u>Group 3</u>	<u>Group 4</u>	<u>Group 5</u>
Stored unpeeled at 0-1°C	Stored peeled at 0-1°C	Stored peeled at 0-1°C	Blanching 2 min, stored unpeeled at 0-1°C until peeling and irradiation 1×10^5 rads	Commercially pre-cooked and brine-dipped shrimp
Untreated control		Pre-irradiation 0.6×10^5 rads Re-irradiation 2×10^5 rads	Peeling and irradiation immediately after blanching	
Pre-irradiation 0.6×10^5 rads		Blanching 2 min, irradiation 1×10^5 rads	Peeling and irradiation 2 days after blanching	Peeling and irradiation 2×10^5 rads 3 days after pre-cooking
Blanching 2 min	Blanching 2 min	Blanching 2 min irradiation 2×10^5 rads	Peeling and irradiation 5 days after blanching	Peeling and irradiation 2×10^5 rads 6 days after pre-cooking
Blanching 5 min	Blanching 5 min	Blanching 5 min, irradiation 1×10^5 rads	Peeling and irradiation 7 days after blanching	

TABLE III. LOG NUMBER OF SURVIVING BACTERIA AFTER VARIOUS EXPERIMENTAL TREATMENTS OF SHRIMP PRIOR TO FINAL STORAGE AT 0-1°C

	Log number survivors	Log cycle variation		Overall log cycle decrease
		Decrease	Increase	
<u>Group A</u>				
1) Unirradiated (0 rad)	6.11			
2) Blanched 2 min in shell	1.48	4.63	-	4.63
3) Irradiated 0.6×10^5 rads in shell	5.52	0.59	-	0.59
4) Irradiated 0.6×10^5 rads in shell kept 4 days and 2×10^5 rads	4.40	1.12	-	1.71
5) Blanched 2 min, kept 4 days and 2×10^5 rads	1.60	0.48	-	5.11
<u>Group B</u>				
1) Unirradiated (0 rad)	6.22			
2) Blanched 2 min in shell	3.21	3.00	-	3.00
3) Blanched 2 min in shell, peeled and irradiated 1×10^5 rads	1.30	1.90	-	4.92
<u>Group C</u>				
1) Unirradiated (0 rad)	5.60			
2) Blanched 2 min in shell	1.48	4.12	-	4.12
3) Blanched 2 min in shell, kept 2 days	1.78		0.30	
4) Blanched 2 min in shell, kept 2 days, peeled and irradiated 1×10^5 rads	1.30	{ 0.18 0.48		4.30
5) Blanched 2 min in shell, kept 5 days	2.70		1.22	
6) Blanched 2 min in shell, kept 5 days, peeled	4.43	-	2.95	
7) Blanched 2 min in shell, kept 5 days, peeled and irradiated 1×10^5 rads	1.30	0.18	-	4.30
8) Blanched 2 min in shell, kept 7 days	4.10	-	2.62	
9) Blanched 2 min in shell, kept 7 days, peeled	3.10	-	1.62	
10) Blanched 2 min in shell, kept 7 days, peeled and irradiated 1×10^5 rads	1.48	0	-	4.12
<u>Group D</u>				
1) Unirradiated (0 rad)	5.39	-	-	
2) Blanched 2 min in shell	1.60	3.80	-	3.80
3) Blanched 5 min in shell	1.00	4.40	-	4.40
4) Blanched 2 min in shell, peeled	3.85	(1.54)	2.25	
5) Blanched 2 min in shell, peeled and 1×10^5 rads	2.56	1.30	0.96	2.83
6) Blanched 2 min in shell, peeled and 2×10^5 rads	2.28	1.57	0.68	3.10

	log number survivors	Log cycle variation		Overall log cycle decrease
		Decrease	Increase	
<u>Group E</u>				
1) Unirradiated (0 rad)	4.97	-	-	
2) Blanched 5 min in shell	1.30	-	-	3.67
3) Blanched 5 min in shell, kept 2 days, and peeled	3.34	1.63	2.14	
4) Blanched 5 min in shell, kept 2 days, peeled and 1×10^5 rads	1.00	{ 0.30 2.34	-	3.97
<u>Group F</u>				
1) Commercially pre-cooked in shell	3.68	-	-	-
2) Commercially pre-cooked in shell, peeled	3.34	-	-	-
3) Commercially pre-cooked in shell, peeled and 2×10^5 rads	1.00	-	-	2.68
4) Commercially pre-cooked in shell, kept 3 days	4.38	-	0.70	-
5) Commercially pre-cooked in shell, kept 3 days, peeled and 2×10^5 rads	1.48	2.90	-	2.20

pasteurization doses of irradiation and heat treatment (blanching) was determined either as a single factor or more often in combination. The results are shown in Table III.

The summary of results of the storage life of shrimp at $0-1^\circ\text{C}$ at the end of various treatments as judged by the total bacterial count (TBC), sensory evaluation score (T-test) and trimethylamine (TMA) value, is shown in Table IV.

The T-test was used as a basis of comparison with the other two tests. The TBC is reported at a borderline of acceptability at both 1×10^6 and 1×10^7 bacteria per gram of sample and showed a fairly close agreement with the T-test on untreated or blanched-only shrimp. However, at times it showed a somewhat shorter storage life of product which had received the combined treatment of blanching and irradiation. The TMA-test appears to be unreliable as a terminal index of storage life of blanched, irradiated shrimp.

The results showed that low-dose pre-irradiation of unpeeled shrimp was ineffective in preventing discolouration and delaying spoilage. Higher dose levels of irradiation alone were also found ineffective in preventing discolouration of shrimp in shell or peeled shrimp. Results showed that unpeeled shrimp blanched for two minutes could be kept for about one week at $0-1^\circ\text{C}$. They could be kept for a longer period of time when, before peeling and irradiation, five minutes blanching was used. The post-irradiation storage life

TABLE IV. STORAGE LIFE OF SHRIMP AFTER VARIOUS TREATMENTS; STORAGE TEMPERATURE 0-1°C

Sample treatment before storage	TBC $\geq$ 1×10^6 after days ^a	T-test ≤ 2 after days	TMA at end of experi- ment
<u>Group 1: stored unpeeled</u>			
Untreated control	7	5	5
Pre-irradiated with 0.6×10^5 rads	-	6	1
Blanched 2 min	10 (11)	11	8
Blanched 5 min	12 (14)	17	4
<u>Group 2: stored peeled</u>			
Blanched for 2 min	13 (14)	14	0.5
Blanched for 5 min	12 (14)	16	0.5
<u>Group 3: stored peeled</u>			
$0.6 \times 10^5 + 2 \times 10^5$ rads	18 (19)	5	1
Blanched 2 min + 1×10^5 rads	21 (25)	41	4
Blanched 2 min + 2×10^5 rads	34 (39)	42	1
Blanched 5 min + 1×10^5 rads	41 (45)	46	1
<u>Group 4: stored blanched, unpeeled until irradiation</u>			
Blanched 2 min + 1×10^5 rads at 0 days	21 (25)	41	4
Blanched 2 min + 1×10^5 rads after 2 days	31 (34)	42	0.5
Blanched 2 min + 1×10^5 rads after 5 days	31 (40)	40	10
Blanched 2 min + 1×10^5 rads after 7 days	26 (32)	35	1

^a Values in parenthesis are TBC when borderline of acceptability is raised to 1×10^7 .

was not affected adversely. Blanching (or pre-cooking) is a necessary pre-irradiation treatment in order to delay spoilage before irradiation and, furthermore, to delay or prevent discolouration of the irradiated product.

The dose level of choice for peeled shrimp appears to be below 2×10^5 rads, as even at and above that dose level undesirable organoleptic changes occur. Doses used on the blanched product were 1×10^5 and 2×10^5 rads, but a dose of 3×10^5 rads was found objectionable to some panelists. From

TABLE V. SUMMARY TABLE OF STORAGE LIFE IN DAYS OF COD FILLETS UN-IRRADIATED AND IRRADIATED AT A DOSE LEVEL OF 2×10^5 RADS; STORAGE TEMPERATURES 0-1°C AND 5-6°C

Storage temperature	Dose level of irradiation	Origin of cod fillets	O-test, date of rejection (days)	TMA ≥ 5 mg N/100 g (days)	TBC $\geq 10^7$ (days)
5-6°C	0 rad	Line cod	3	2	2
"	"	Net cod	4	2	2
"	"	Trawl cod	4	2	2
0-1°C	"	Line cod	7	6	6
"	"	Net cod	5	4	5
"	"	Trawl cod	5	4	5
5-6°C	2×10^5 rads	Line cod	13	11	8
"	"	Net cod	11	9	7
"	"	Trawl cod	11	8	6
0-1°C	2×10^5 rads	Line cod	24	22	14
"	"	Net cod	20	16	12
"	"	Trawl cod	19	17	11
0-1°C	2×10^5 rads	Line cod, special lab. filleting	33	31	26

the bacterial standpoint, a dose level of 2×10^5 rads is desirable. The five-minutes blanching plus irradiation at a dose level of 1×10^5 rads gives somewhat longer storage life. The longer blanching time (five minutes) was not tested as extensively as the two-minutes blanching time.

By using the optimal combination of treatments which is felt to be five-minutes blanching and irradiation at a dose level of 1×10^5 rads a storage life of peeled shrimp was found to be six to seven weeks.

3.3. Results on the study of radiation pasteurization of whole cod and cod fillets

Table V gives a summary of storage life in days of cod fillets unirradiated and irradiated at a dose level of 2×10^5 rads, stored at $0-1^\circ\text{C}$ and $5-6^\circ\text{C}$.

Whitefish (e.g. cod and haddock) are caught in Icelandic waters mainly by three methods: by line, by gill-netting and by trawling. Of the three methods, the line fisheries give the best quality fish, the trawl fisheries give fish of more variable quality, whereas the net fisheries yield fish of the lowest quality.

The aim of this study was to investigate the effect of the raw material quality of cod on the quality of the irradiated product and its effect on the storage-life extension. This is known to affect also the quality of iced and frozen fish. The difference in quality between line-caught fish and net-caught fish lies in the catching method rather than in the time elapsed from capture until processing begins. For the trawl fish, however, the time of storage on ice is the limiting factor for quality.

The commercial fillets used in this study were of known past history when received at the laboratory from the freezing plant. This includes information on the method and time of catching and the period of storage before and after landing.

To evaluate the initial quality and the quality during storage, four test criteria were used as outlined in sections 2.6 and 2.7 above.

The O-test is a convenient and reliable method of evaluating the quality of unprocessed whitefish, which is of great value to a fish-exporting country.

Of the chemical tests, the VAN proved to be of limited value for irradiated cod fillets. In this study, no correlation was found between the VAN and the other three test criteria, for samples irradiated with 2×10^5 rads.

On the other hand, TMA-values showed fairly good agreement with the O-test and the TBC, lying in between the two tests, but closer to the O-test. A borderline TMA-value of 5 mg TMA-N/100 g of fish, proposed by Castel [5], was used in this study.

It has been suggested in the literature that the borderline of acceptability for bacterial counts of irradiated fish be set at a higher scale than 1×10^6 bacteria per gram, which is the limit set for unirradiated, spoiling fish. Bacterial counts of 1×10^7 bacteria per gram have been proposed. In the

TABLE VI. COMPARISON OF STORAGE LIFE OF LINE AND NET-CAUGHT WHOLE COD UNIRRADIATED AND IRRADIATED AT A DOSE LEVEL OF 0.5×10^5 RADS; STORED AT $0-1^\circ\text{C}$

Days of storage	Line cod Control 0 rad				Net cod Control 0 rad				Line cod 0.5×10^5 rads				Net cod 0.5×10^5 rads			
	0-test ^a	VAN	TMA	TBC	0-test ^a	VAN	TMA	TBC	0-test ^a	VAN	TMA	TBC	0-test ^a	VAN	TMA	TBC
0	1.5	7.2	0.6	1.0×10^1	5.0	6.0	0.8	4.0×10^1	1.5	7.2	0.6	0.0	5.0	6.0	0.8	1.0×10^1
3					3.3	8.4	2.4	7.3×10^3					5.5	7.6	0.7	2.0×10^1
5	3.7	6.0	2.8	5.0×10^1					3.5	5.6	0.8	1.0×10^2				
6					10.2r	9.6	8.5	2.8×10^4								
7													7.4	7.2	2.8	2.6×10^3
8					-	26.4	104.0	1.4×10^7	3.4	5.0	0.3	8.0×10^7				
9	8.8	21.2	4.1	1.9×10^4												
12	9.2r	32.4	19.4	1.5×10^5					5.0	7.2	2.9	3.0×10^3				
13													9.2r	11.6	15.1	1.7×10^5
14									5.0	7.2	2.7	5.7×10^3				
15	-	-	-	6.1×10^6									-	20.0	53.2	5.8×10^6
19									6.7	12.4	9.0	5.1×10^4				
21									9.8r	18.8	22.3	5.4×10^4				
23									-	-	50.0	5.3×10^5				
27									-	-	-	2.8×10^7				

^a Values in 0-test columns followed by 'r' give the estimated age in days at time of rejection.

previous studies 1×10^6 and/or 1×10^7 bacteria per gram were used, but in this study 1×10^7 bacteria per gram are used exclusively.

The unirradiated fillets kept at $0-1^\circ\text{C}$ showed close agreement between the three test criteria. With irradiated fillets, the relationship between TBC, TMA and O-test was such that the borderline of acceptability was reached earliest by TBC, then by TMA and finally by the O-test. For irradiated fillets, it was shown that the storage life determined by the O-test, TMA and TBC was dose-dependent. The dose level of 3.0×10^5 rads proved to be unacceptable, but only slight preference could be detected by the panelists between 1.0 and 2.0×10^5 rads.

By using 2.0×10^5 rads, a few days longer storage life was obtained than by 1.0×10^5 rads. The reason for using the 2.0×10^5 rads dose level for irradiating cod fillets in this study is that it was felt that for Icelandic conditions a longer storage-life extension would be necessary for irradiated fish products to be shipped to foreign markets.

Storage life at a temperature of $5-6^\circ\text{C}$ was about one half of that obtained at $0-1^\circ\text{C}$.

By irradiating the three types of cod fillets with 2.0×10^5 rads, the storage life at $0-1^\circ\text{C}$ was extended three to four times beyond that of the control.

The storage life of the line-cod fillets lasted up to 24 days, that of the net and the trawl-cod fillets was 19 and 20 days respectively.

The fillets of line cod showed significantly longer storage life than those of net and trawl cod.

The low-level irradiation of whole, eviscerated cod was tested because a considerable quantity of iced, whole cod is exported to Europe. In the two major importing countries, the United Kingdom and Germany, many customers buy the whole fish in preference to fresh fillets.

The dose level of 0.5×10^5 rads chosen in this study is quite likely to be the lowest effective dose level for significant storage-life extension of whole fish. This dose level has been suggested [6, 7], but is intended for pre-irradiation of whole fish followed by re-irradiation of the fillets at higher dose level (1.0 to 2.0×10^5 rads).

It was felt that the total dose should not exceed 2.0×10^5 rads. It was concluded from the O-tests that the dose level of 0.5×10^5 rads would not cause radiation off-odours even to the most particular fish eaters.

In order to emphasize the importance of the raw-material quality of whole cod (and fillets) to be preserved by irradiation, line and net cod were compared. As pointed out earlier in this paper, the method of catching rather than the age after time of capture, determines the quality of the fish landed.

Table VI shows that the whole net cod, both unirradiated and irradiated, had a storage life which was only about a half of that of the line cod. Furthermore, the net cod gave from the beginning a much worse O-test rating and the appearance (not included in the O-test) was far inferior to the line cod.

Net-cod fillets re-irradiated at 1.5×10^5 rads after six days of storage as whole, pre-irradiated cod, were rejected from the beginning by the panel.

TABLE VII. COMPARISON OF STORAGE LIFE OF WHOLE LINE-CAUGHT COD, PRE-IRRADIATED WITH 0.5×10^5 RADS AND FILLETS THEREOF, RE-IRRADIATED WITH 1.5×10^5 RADS

Whole cod stored 3, 7 and 12 days prior to filleting; samples stored at $0-1^\circ\text{C}$

Days of storage	Whole cod 0.5×10^5 rads				Filleting after 3 days Re-irradiation 1.5×10^5 rads				Filleting after 7 days Re-irradiation 1.5×10^5 rads				Filleting after 12 days Re-irradiation 1.5×10^5 rads			
	0-test ^a	VAN	TMA	TBC	0-test ^a	VAN	TMA	TBC	0-test ^a	VAN	TMA	TBC	0-test ^a	VAN	TMA	TBC
0	1.5	7.2	0.6	0.0												
3					3.2	8.0	0.5	1.0×10^1								
5	3.5	5.6	0.8	1.0×10^2												
7									6.2	8.8	2.0	2.4×10^3				
8	3.4	5.0	0.3	8.0×10^1	6.2	10.0	0.7	1.5×10^2								
12	5.0	7.2	2.9	3.0×10^3									5.0	8.4	2.4	6.0×10^3
14	5.0	7.2	2.7	5.7×10^3	5.8	6.0	0.9	7.0×10^5	7.2	6.8	1.1	3.3×10^4				
16													5.7	9.2	1.2	2.3×10^3
17					6.0	6.4	0.7	2.0×10^7	6.3	6.8	1.2	5.7×10^6				
19	6.7	12.4	9.0	5.1×10^4												
20					8.3	6.4	2.2	3.3×10^7	8.3	6.4	2.2	2.6×10^7	6.0	8.0	2.8	2.0×10^5
21	9.8r	18.8	22.3	5.4×10^4												
23	-	-	50.0	5.3×10^5	10.6r	7.2	3.2	-	8.0	6.4	1.6	-	6.4	7.2	1.8	1.4×10^7
27	-	-	-	2.8×10^7		6.8	25.0	-	10.6r	6.4	9.8	-	8.2	9.6	5.8	9.4×10^7
30									-	-	39.0	-	9.9r	12.0	21.1	-

^a Values in 0-test columns followed by 'r' give the estimated age in days at time of rejection.

From this it was concluded, that net-caught whole cod is unsuited for radiation preservation.

By irradiating the line cod with 0.5×10^5 rads, its storage life could be doubled or extended by ten days.

This storage-life extension is significant, but it should be pointed out that the line-cod control (unirradiated) had a twelve-days storage life, whereas the commercial line-cod fillets had seven-days storage life. The difference in storage life may undoubtedly be attributed to environmental contamination of the fillets.

From the bacteriological viewpoint it is interesting to note that the whole line cod reached the borderline of 1.0×10^7 bacteria per gram at a much later date than the other test criteria (O-test, TMA and VAN) indicated. This is contrary to what was seen with the cod fillets and is probably caused by slower bacterial penetration into the whole fish.

Contrary to the irradiated cod fillets (2.0×10^5 rads), the irradiated whole cod (0.5×10^5 rads) showed an increase in VAN, which is similar to that found in unirradiated cod during storage.

The TMA of whole cod behaved similarly to what was found in the study of the cod fillets.

One experiment was conducted in this study by irradiating whole line cod (pre-irradiation), filleting of the whole cod after 3-, 7- and 12-days storage and re-irradiating the fillets by 1.5×10^5 rads on the days of filleting. From Table VII. it may be seen that post-irradiation storage life of the fillets cut from pre-irradiated whole cod appears to be inversely related to the storage time of the pre-irradiated whole cod, i.e. the longer the whole cod was stored, the shorter was the post-irradiation storage life of the fillets. Further, that the total storage life of re-irradiated cod fillets is only slightly longer than that of the whole, pre-irradiated cod: The total bacterial counts of the fillets reached the borderline of 1.0×10^7 per gram for samples taken underneath the skin from the whole cod.

The effect of the environmental contamination was repeatedly noted in the various experiments. This led to setting up an experiment where the effect of the sanitary care in handling during preparation (filleting, etc.) was evaluated. The results of this experiment showed these factors very clearly. By using special care in sanitation in filleting in the laboratory, storage-life extension of nine days was found beyond that of the commercial machine-cut cod fillets.

The importance of sanitation during preparation of fish products that are to be preserved by irradiation must be strongly emphasized.

To summarize the main conclusion drawn from the study on radiation pasteurization of whole cod and cod fillets, it may be said that as very fresh raw material, line-caught cod and haddock, is always available in Iceland, radiation preservation should be performed in a shore-based radiation centre. This shore radiation centre could also process other seasonal seafoods. The filleting of cod should be done by machine and strict sanitary measures used during handling and packing prior to irradiation. The dose level for irradiation of cod fillets recommended in this study was 2×10^5 rads. Low-level radiation of whole, eviscerated cod showed a promise for export to foreign markets.

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RADURIZATION OF FRESH-WATER FISH IN THE FEDERAL REPUBLIC OF GERMANY

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Abstract

RADURIZATION OF FRESH-WATER FISH IN THE FEDERAL REPUBLIC OF GERMANY.

Fresh gwyniad, trout and carp were vacuum-packed in plastic foil impermeable to gas and water vapour, irradiated with 10-MeV electrons, and stored under melting ice. Sensory, chemical and microbiological tests were then carried out over a period of four to two weeks. In the case of gwyniad, best results were obtained with a dose of 0.1 Mrad which prolonged Grade-1 quality shelf life from nine to 23 days. With trout a dose of 0.05 Mrad improved the shelf life from 15 to 21 days. Doses higher than 0.1 Mrad for gwyniad and 0.05 Mrad for trout caused a noticeable loss of flavour. In this respect trout appears to be particularly radiation-sensitive in comparison with other species of fish. In contrast, carp turned out to be particularly well suited for radiation-preservation treatments, as its flavour is not negatively affected even by relatively high radiation doses. While unirradiated carp could be stored for 15 days, a dose of 0.5 Mrad preserved the Grade-1 quality shelf life for 35 days. Higher doses, up to 1.5 Mrad, caused a further reduction of microbial counts without improving the shelf life beyond the results obtained with 0.5 Mrad. The latter dose, which does not affect flavour, can therefore be considered optimal for carp.

1. INTRODUCTION

The production of fresh-water fish has considerable importance in several areas of the Federal Republic of Germany. Carp and trout are raised in many fish farms, while gwyniad (Blaufelchen) are caught in Lake Constance and in some Bavarian lakes. The main season for carp is around Christmas and New Year. The demand is so great at this time that it can be met only by additional imports from France and Yugoslavia. Carp are traded and shipped live and are slaughtered in the fish store when they are sold to the consumer. Trout are sold either frozen or live. Gwyniad are usually consumed fresh as a local specialty near Lake Constance.

Irradiation could bring a number of advantages both to the fish industry and to the consumer. Improvement in shelf life would make it unnecessary to trade and keep living carp and trout in tanks. The fish could be slaughtered in the fish farm and transported under melting ice. Also because of improved shelf life, gwyniad could be transported without loss of quality to places more remote from Lake Constance.

In comparison to the numerous publications on irradiation of ocean fish there have been only a few investigations on radiation preservation of fresh-water fish [1—8]. It seemed desirable, therefore, to study the possibilities of irradiating fresh-water fish farmed or caught in the Federal Republic of

Germany. In view of the fact that the methods of preparation and consumer expectations may be quite different in different countries, sensory evaluation was considered to be a very important aspect of these studies. In the present paper we wish to summarize and to compare the results on the radurization of fresh-water fish which we have obtained on gwyniad [9], trout [10] and carp [11].

2. MATERIAL

Carp and trout were obtained from a fish farm nearby. The fish were slaughtered and gutted there and immediately packed individually under 95% vacuum in laminated foil (Hostaphan PE). The fish were transported to the laboratory at Karlsruhe under melting ice and irradiation was carried out the same day. Gwyniad were caught in Lake Constance in the morning, gutted on the boat, stripped of scale immediately after landing and also packed individually under 95% vacuum in the above-mentioned foil. During transport to Karlsruhe and subsequent storage the fish were covered with melting ice. Gwyniad were irradiated the day after they were caught.

The electron linear accelerator at the institute served as the radiation source. The energy of the electrons was 10 MeV, the dose rate of the electron pulse was 10^{10} rad/s. The fish were irradiated in open trays without ice. The total irradiation time, including transport over the conveyer belt, was approximately two minutes so that the fish could not warm up significantly during this time. After irradiation the fish were again placed under melting ice, together with a number of unirradiated samples. In addition, some unirradiated samples were deep frozen to serve as controls in the sensory evaluation.

3. SENSORY EVALUATION

The fish were cooked individually and their quality was judged by a trained panel of eight. A deep-frozen sample was always served as a control. No more than six samples were served in one session. A ten-point scale was used, "9" indicating best quality, "1" indicating total spoilage. The colour of the skin and meat, odour, taste and texture were judged. The panelists did not know which samples had been irradiated and which were not. Only the deep-frozen control sample was identified. The panelists agreed on a uniform grade for this sample in order to make their evaluation comparable. No consistent effect of storage or irradiation on the colour of the skin or the colour of the meat was observed. The scores given for odour were so similar to those given for taste that in this paper we present only the taste scores.

It can be seen from Figs 1 to 3 that the taste scores were about the same during the first two weeks of storage, independent of radiation dose. Only on the first day, e.g. immediately after irradiation, decreasing taste scores were given with increasing radiation dose, most notably in the case of trout.

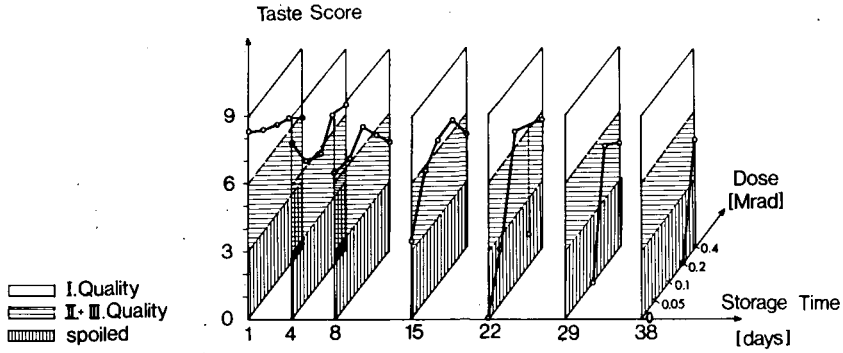


FIG. 1. Sensory evaluation of Felchen (gwyniad) during storage on ice after irradiation.

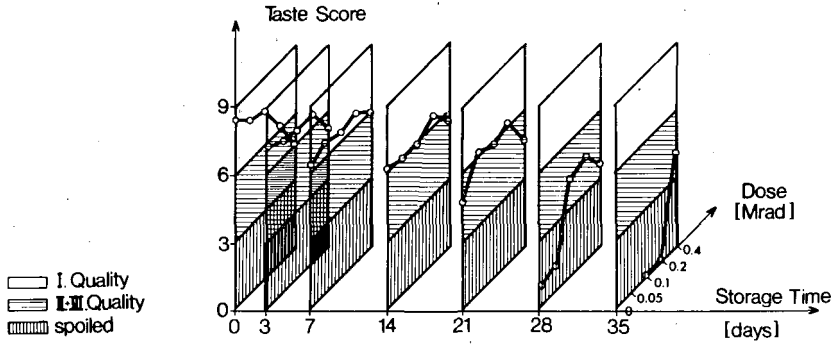


FIG. 2. Sensory evaluation of trout during storage on ice after irradiation.

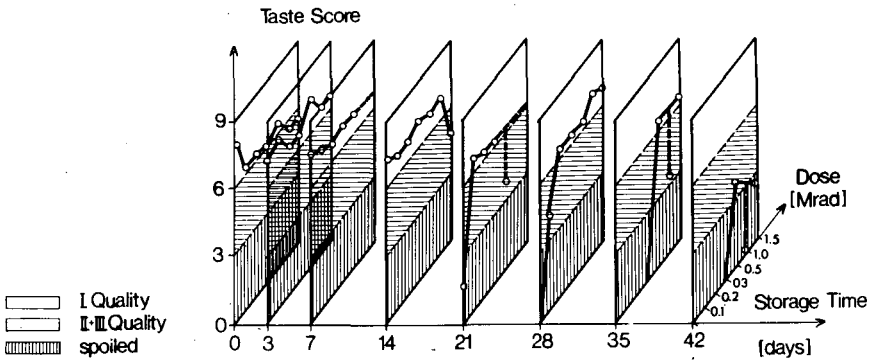


FIG. 3. Sensory evaluation of carp during storage on ice after irradiation.

After two to three weeks of storage a rapid fall in taste scores indicated spoilage, first in unirradiated samples, then in those irradiated with a low dose, and finally in those irradiated with a high dose.

If we consider the decline of quality to below Grade 6 as the end of shelf life and below Grade 3 as the beginning of spoilage, we obtain the results presented in Table I. With increasing radiation dose an increasing improvement of shelf life was achieved. In the case of gwyniad and trout, however, advantage cannot be taken of this improvement above 0.1 Mrad because of the loss of flavour which is observed at higher doses. In contrast to the observations of other authors [3], a certain loss of flavour was already observed with trout at 0.05 Mrad during the first few days after irradiation; this was more pronounced at 0.1 Mrad. A similar loss of flavour was observed in the deep-frozen trout which served as controls, when compared to the fresh trout which had been tested on the first day of the experiment. Doses of 0.2 and 0.4 Mrad caused a foreign flavour in trout, which disappeared in the course of one week of storage. A "radiation flavour" of this kind was not found in gwyniad. In agreement with the observations of Kardashev [2], our panel did not observe a radiation flavour in carp, even after irradiation with a dose of 1.5 Mrad. Carp has a much stronger natural flavour than trout and gwyniad and we assume that this covers the radiation flavour which is to be expected at high doses. Some members of the panel particularly liked the taste of carp irradiated with the highest dose. It appeared that the radiation treatment enhanced the typical carp flavour.

TABLE I. SENSORY EVALUATION OF SHELF LIFE ON ICE AFTER RADURIZATION

Dose (Mrad)	Felchen (gwyniad)	Trout		Carp		
	Storage time (days) up to:					
	Loss of Grade-1 quality	Spoilage	Loss of Grade-1 quality	Spoilage	Loss of Grade-1 quality	Spoilage
0	9	16	15	24	15	20
0.05	12	20	21	26		
0.1	23	26	21	30	23	30
0.2	(23) ^a	32	22	31	28	32
0.3					29	32
0.4	(23) ^a	> 38	(22) ^a	> 35		
0.5					36	43
1.0					30	36
1.5					36	41

^a These samples did not reach Grade-1 quality over the duration of the experiment because of impairment of flavour.

An effect of irradiation on texture was not observed. A certain softening of the meat occurred after long periods of storage, particularly in carp which had been irradiated with 0.5 and 1.5 Mrad and stored for five to six weeks. It is well known that the radiation doses used in our experiments were insufficient to inactivate enzymes [12] and the effects observed on texture may well be considered as signs of enzymatic spoilage. The decrease in taste scores for irradiated trout and gwyniad after three weeks of storage and for carp after five weeks of storage is probably also related to enzymatic effects. With trout and gwyniad an improvement of shelf life beyond this time cannot be achieved by applying higher radiation doses because of the loss of flavour mentioned above which is caused by higher doses. With carp, higher doses would be possible but they would not lead to a very long shelf life, unless enzymes were inactivated by an additional blanching process, as suggested for fish patties [13] and for cod fillets [14].

Based on sensory evaluation, a dose of 0.5 Mrad can be considered as optimal for carp. It preserves a Grade-1 quality for 35 days instead of 15 days without irradiation. With gwyniad, 0.1 Mrad was optimal, giving a Grade-1 quality shelf life of 22 days instead of eight days, while with trout the maximum dose that can be applied without causing loss of flavour is 0.05 Mrad, causing an extension of Grade-1 quality shelf life from 15 to 26 days.

4. CHEMICAL QUALITY CONTROL

Basic nitrogen was determined by the method of Antona [15]. With gwyniad and carp the sample was taken from the side of the fish, with trout from the muscles of the back. The trimethylamine (TMA) content of trout was

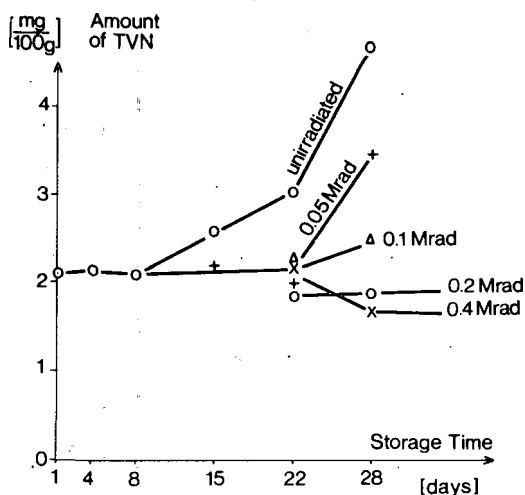


FIG. 4. Chemical evaluation of Felchen (gwyniad) during storage on ice after irradiation; total volatile nitrogen (TVN).

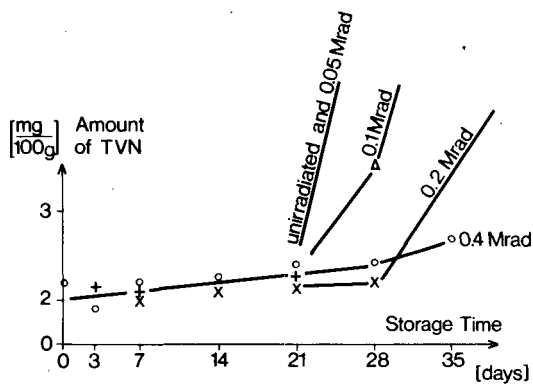


FIG. 5. Chemical evaluation of trout during storage on ice after irradiation; total volatile nitrogen (TVN).

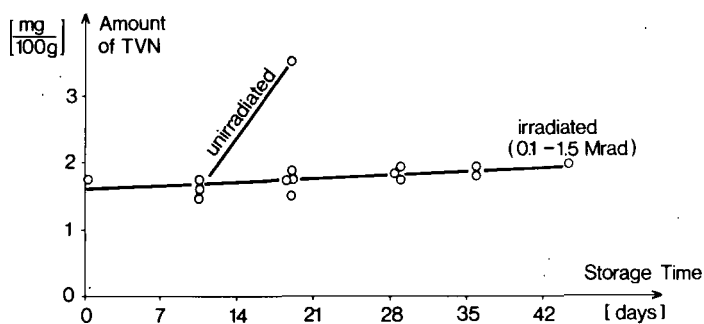


FIG. 6. Chemical evaluation of carp during storage on ice after irradiation; total volatile nitrogen (TVN).

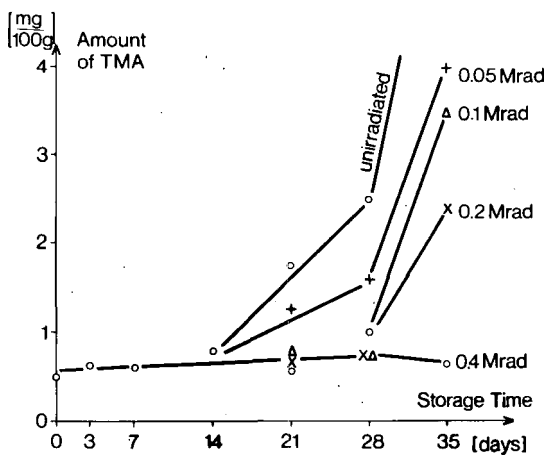


FIG. 7. Chemical evaluation of trout during storage on ice after irradiation; trimethylamine (TMA).

also determined by a method described by Antona [15]. While endogenous trimethylamine oxide does not occur in fresh-water fish, the production of TMA in stored trout is not unusual, due to the use of fish meal for feeding farmed trout [16]. The results presented in Figs 4 to 7 indicate that incipient spoilage was recognized by sensory evaluation before it was clearly indicated by chemical methods. In agreement with other authors [17—19] we find that the use of chemical quality-control tests is even more difficult in irradiated fish than in unirradiated. It may also be mentioned that chemical criteria for spoilage of fresh-water fish have been studied much less than those for ocean fish [16].

The discrepancy between the results of chemical tests and sensory evaluation was particularly pronounced with gwyniad. Only a slight increase in total volatile basic nitrogen (TVN) was found in samples which were judged as spoiled by the sensory test. With trout on the other hand the increase of TMA and TVN correlated quite well with the onset of spoilage as judged organoleptically. However, even in this case loss of Grade-1 quality (taste score below 6) occurred before chemical indicators changed. With irradiated carp no development of TVN was found during the six-week storage period regardless of radiation dose, while in unirradiated samples it developed strongly after two weeks of storage.

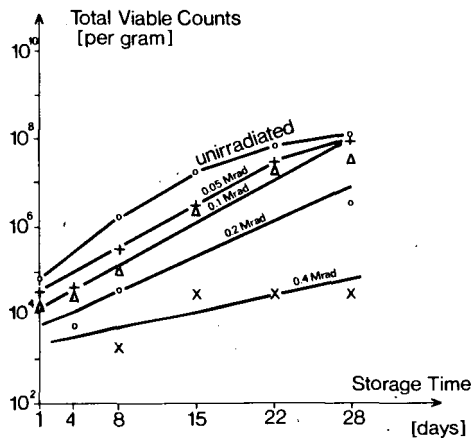


FIG. 8. Microbiological evaluation of Felchen (gwyniad) during storage on ice after irradiation.

5. MICROBIOLOGICAL EVALUATION

Total microbial counts were determined parallel to chemical and sensory testing. For this purpose, five samples of muscle tissue were taken from one fish and homogenized after addition of peptone (0.1%)-sodium chloride (0.85%) solution. After appropriate dilution of the homogenate, plates were prepared using casein peptone-yeast extract-glucose-agar.

Total viable counts for fish treated with various radiation doses and stored for various periods of time in melting ice are presented in Figs. 8 to

TABLE II. MICROBIOLOGICAL EVALUATION OF SHELF LIFE ON ICE AFTER RADURIZATION

Dose (Mrad)	Felchen (gwyniad)	Trout		Carp		
	Storage time (days) up to total viable counts of:					
	10 ⁶ /g	10 ⁷ /g	10 ⁶ /g	10 ⁷ /g	10 ⁶ /g	10 ⁷ /g
0	6	12	12	17	11	15
0.05	11	17	18	27		
0.1	14	21	21	30	20	(> 36) ^a
0.2	18	26	24	30	23	(> 36) ^a
0.3					25	(> 36) ^a
0.4	(> 28) ^a	(> 28) ^a	28	(> 35) ^a		
0.5					27	(> 36) ^a
1.0					(> 36) ^a	(> 36) ^a

^a Over the duration of the experiment these samples did not reach total viable counts of 10^6 /g or 10^7 /g, respectively.

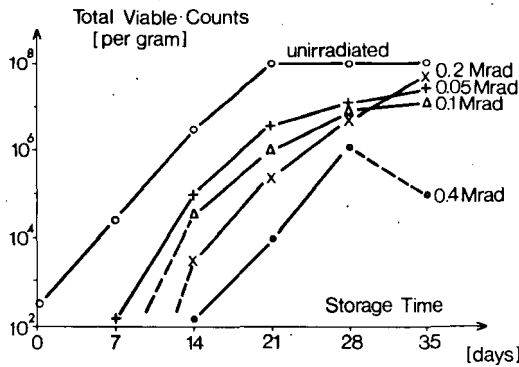


FIG. 9. Microbiological evaluation of trout during storage on ice after irradiation.

10. The initial microbial count of trout was remarkably low, approximately 500 counts/g, and much higher for carp and gwyniad (10^4 – 10^5 /g). Irradiation greatly reduced these numbers, usually to less than 100/g with a dose of 0.1 Mrad or higher. In Figs 9 and 10 this is indicated by the broken-line extrapolation of the microbial counts.

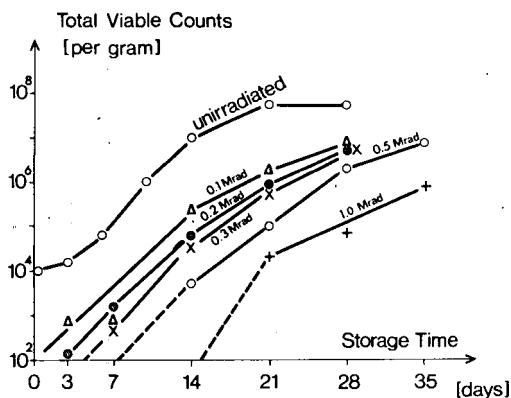


FIG. 10. Microbiological evaluation of carp during storage on ice after irradiation.

Table II indicates how increasing radiation doses delay the occurrence of microbial counts which are typical for spoiled products. A comparison of Table II with Table I shows that 10^6 counts/g were reached at a time when a sensory evaluation showed loss of Grade-1 quality, while 10^7 counts/g were reached when the sensory evaluation indicated spoilage.

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PRESERVATION BY GAMMA RADIATION OF BOMBAY DUCK, SHRIMPS AND WHITE POMFRETS

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Abstract

PRESERVATION BY GAMMA RADIATION OF BOMBAY DUCK, SHRIMPS AND WHITE POMFRETS.

Radiation-processing procedures have been developed for extending storage shelf life at sub-room as well as room temperatures for Bombay duck (*Harpodon nehereus*), shrimps (*Metapenaeus* sp. and *Parapeneopsis stylifera*) and white pomfrets (*Stromateus cinereus*). The physical, chemical and microbiological changes in each of these seafoods have been evaluated critically to establish the technological merits of the processes. Studies on the radiation sensitivity and biochemical properties of fish microflora provide evidence for the selective control of rapid spoilers in irradiated Bombay duck. The biochemically inert nature of *Micrococcus* sp. predominant in radiation-pasteurized Bombay duck concurrent with the suppression of Gram-negative spoilers, brings about a high acceptability of irradiated products during storage. Irradiation at 0.1 Mrad extends the storage life of Bombay duck for 18–20 days at 0–2°C due to a lower spoilage rate as against the rapid deterioration of unirradiated samples within 5–7 days. Textural losses due to exudation of drip in irradiated as well as in unirradiated Bombay duck can be minimized by pre-dip treatments in 10% NaCl for 60 min or in 10% sodium tripolyphosphate for 15 minutes. Fresh Bombay-duck fillets or laminates can be preserved by a radiation dose of 0.25 Mrad for three weeks at 0–2°C and partially dried laminates for three months at 28–32°C. Insect damage to air-dried or commercially sun-dried Bombay-duck laminates can be prevented by 15 krad. The tolerance dose in the range 0.15–0.25 Mrad for shrimps has been selected on the basis of retaining the naturally occurring pigment and the absence of irradiation odours. The radiation-pasteurization process for fresh and blanched shrimps (100°C, 4 min) extends the shelf life by 18 and 130 days respectively at 0–2°C. Cooked and semi-dried shrimps can be stabilized for room-temperature storage for 3 and 6 months respectively. The development of rancid odours and yellow discolouration during the storage of irradiated pomfret (above 0.3 Mrad) can be effectively minimized by maintaining anaerobic conditions during irradiation and storage. The radiation-pasteurization process for white pomfret fillets consists of anaerobic packaging and irradiation (0.1 Mrad). This treatment augments the shelf life of pomfrets by 35 days at 0–2°C as against the spoilage of unirradiated products within 7 days. Transportation studies of irradiated Bombay duck, eel and shrimps show prospects for radiation-pasteurization processing in the inland distribution of fishery products.

1. INTRODUCTION

In recent years there has been considerable progress in efforts at harnessing the fishery potential of the seas in India. A steady transformation from the traditional to modern methods of fishing and processing is taking place. With an annual production of over a million tons, India is among the seven major fish-producing countries of the world. The income from fisheries

TABLE I. PERCENTAGE OF FROZEN, CANNED AND DRIED SEA FOODS AND OTHER MISCELLANEOUS ITEMS IN THE TOTAL EXPORT OF MARINE PRODUCTS FROM INDIA FOR THE YEARS 1963 TO 1968^a

Year	Frozen		Canned		Dried		Other		All
	Qty (%)	Value (%)	Qty (%)	Value (%)	Qty (%)	Value (%)	Qty (%)	Value (%)	
1963	25.38	42.19	6.87	12.92	66.20	44.50	1.55	0.39	100
1964	29.11	48.99	5.01	10.20	63.19	40.4	2.69	0.41	
1965	49.11	65.6	7.43	13.73	41.26	20.22	2.20	0.45	
1966	49.20	70.88	7.95	13.80	41.02	14.68	1.83	0.64	
1967	55.55	70.75	10.11	15.68	32.08	13.00	2.26	0.57	
1968	61.97	76.06	9.02	11.84	28.74	11.77	1.77	0.33	

^a Source: Indian Sea Foods, Vol. VII, No. 1 (1969—70).

TABLE II. EXPORT OF MARINE PRODUCTS FROM INDIA DURING THE YEARS 1966—67, 1967—68, 1968—69 (IN TONNES)^a

	1966—67	1967—68	1968—69
Frozen shrimp	9 838	11 856	16 315
Canned prawns	1 959	2 054	2 166
Dried shrimp	1 196	1 928	1 076
Frozen fish	2	3	.9
Dried fish	6 672	4 567	5 374

^a Source: Indian Sea Food Journal, Vol. VII, No. 1 (1969—70).

for 1966 was estimated at \$80-million, with annual foreign-exchange earnings of over \$17-million. India exports a variety of seafoods including frozen, canned and dried shrimps, frozen lobster tails, frozen frog legs and dried fish. Frozen and canned shrimps are exported to the United States of America, Australia, Japan and European countries. The good demand for dried shrimp and fish from Ceylon, the Far-East, Middle-East countries and Africa is being met by the export of large quantities of sun-dried products.

The drastic changes taking place in the nature of the export trade in marine products [1] is evident from Table I. During 1962—63, dried fishery products constituted 66% of the total quantity exported, while the corresponding figure for canned and frozen fish products was about 32%. In 1968, however, the former was only 28% whereas the frozen and canned products showed a substantial rise to 70%. Of the total exports, shrimps contribute about 85% by value in one form or another. Table II shows the export values

for shrimps compared to other seafoods during the consecutive years, 1966 to 1969. This clearly points to the dynamic growth of the freezing and canning sectors of the industry.

Despite the progress in the fishery trade for the export market, utilization of sea foods for local consumption is far from satisfactory. On a per-capita basis, this is only about 2 kg in India as against 32.9, 11.4, 61.3 and 49.4 kg reported, respectively, for Korea, USSR, Norway and Japan. Several factors account for this discrepancy. The fishery industry is mainly located on the west coast of India which accounts for three quarters of the total production. The transportation of fish in a fresh form poses serious problems for inland distribution. Frozen seafoods require the maintenance of freezing units from the processing plant to the retailer's market; such facilities are, as yet, inadequate. Due to indifferent methods of handling and processing, besides prevailing unhygienic practices, fish transported in ice often does not reach its destination in a marketable quality. During a period of glut, considerable quantities become spoiled from a lack of adequate processing and storage facilities. Commercial sun-drying of salted or unsalted fish operates only at a cottage-industry level. Due to storage conditions at high humidity and temperature, there are serious problems of insect infestation resulting in heavy losses. Besides, infections with Salmonella and other entero-bacterial pathogens are also favoured under these conditions.

2. SCOPE FOR RADIATION PRESERVATION

Recognizing the need for enlarging the scope for utilizing marine foods and the benefits from an extension of shelf life obtained by radiation-processing procedures, programs of research and development have been in progress at the Bhabha Atomic Research Centre, Trombay, during the last few years.

These programs have been pursued with two objectives:

- (i) To develop radiation-pasteurization procedures for tropical seafoods, especially those which have defied other methods of preservation.
- (ii) To use an appropriate combination of conventional preservation techniques with irradiation so as to minimize the limitations of either method, thereby improving product quality and extending shelf life.

Some of the significant results obtained and pilot facilities in operation at Trombay were reviewed at the Karlsruhe symposium [2] and at a symposium on Science and India's Food Problems [3]. In respect of Bombay duck, detailed technical papers relating to the radiation-pasteurization process [4], the selective control of microflora by gamma radiation [5,6], textural stability [7], chemical and physical changes in irradiated Bombay duck [8] have been reported from this laboratory. Special problems encountered in the radiation-pasteurization of tropical shrimps [9] and the stability of semi-dried shrimps by the dehydro-irradiation process [10] have also been studied. Recently, these aspects were discussed at the Food Irradiation seminar [11,12] organised jointly under the auspices of BARC/FAO/IAEA.

This paper summarizes some of the significant results in respect of Bombay duck, shrimps and pomfrets, emphasizing the combined physical and chemical treatments for enhancing product quality. Process variables have been critically evaluated in terms of the retention of organoleptic attributes [13], freshness indices — total bacterial load and associated biochemical parameters [14], oxidative changes [15] and the nature of predominant bacterial groups [5,6]. Observations on transportation studies of irradiated seafoods are also presented.

3. BOMBAY DUCK

3.1. Problems in preserving Bombay duck

Bombay duck contributes almost 10% of the total catch of fish. During 1968, out of the total of 889 651 tonnes of marine fish landed, Bombay duck represented 77 363 tonnes, pomfrets, 17 845 tonnes and shrimps 91 014 tonnes. Hitherto, Bombay duck has been preserved mostly by sun-drying. For example, in Maharashtra State, during 1967–68, out of the 49 201 tonnes landed, 85% was sun-dried. Because of high free-water content and labile tissue proteins, this fish cannot be canned or frozen. Ice-storage does not extend the storage life beyond 3–7 days, because of rapid spoilage by psychrophilic organisms. Some of the problems encountered in preservation of this fish are given in Table III. The radiation-pasteurization (radurization) process for this fish in fillets and in a laminated form was therefore studied.

3.2. Radiation sensitivity of fish microflora

The radiation-pasteurization process does not reduce all microbial groups in a fixed proportion for a particular radiation dose due to differences in radiation sensitivities, repair mechanisms and dose modifying factors [16–19]. Figure 1 shows the enormous differences in D_{10} values and pattern of inactivation of some of the Gram-negative and Gram-positive organisms [20]. Pseudomonas, Proteus vulgaris, Aeromonas hydrophila and Achromobacter show an exponential relationship, while Micrococcus luteus and Micrococcus sp. (unidentified) isolated from Bombay duck exposed to 0.5 and 1.5 Mrad respectively show an initial shoulder followed by an exponential decline. In this respect, they are similar to M. radiodurans.

The exponential and sigmoidal nature of radiation-survival curves have been ascribed to single and multiple hits, resulting in lethal damage. The magnitude of the shoulder of the survival curve of Micrococci thus indicates the extent of accumulation of hits prior to their manifestation in lethal damage. Several compositional and physical factors have been thought to be responsible for conferring the exceptionally high radiation resistance in M. radiodurans, such as the presence of carotenoid pigments [21], high amounts of unsaturated fatty acids [22], free radical scavengers like mercapto-ethylamine

TABLE III. PROBLEMS IN PRESERVING BOMBAY DUCK

Process	Defects
Refrigeration (5—10° C)	Short shelf life: 2—3 days
Ice-storage (0— 2° C)	Limited shelf life: 5—7 days
Deep-freezing (-20° C)	Tissue damage: drip loss
Thermal-treatment	Tissue damage: drip loss, cooked flavour
Sun-drying	Slow process, poor reconstitution, loss of texture and flavour, browning, poor all-round quality.

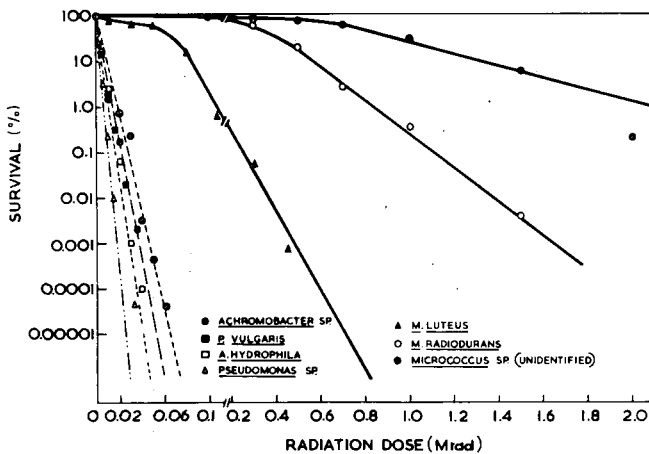


FIG. 1. Radiation sensitivity of fish microflora: organisms from 24-hour broth culture were suspended in a phosphate buffer (pH 7.0) and irradiated; counts of surviving micro-organisms in suspension were determined by the pour-plate technique.

[23], etc. The unique ultra-fine structure of the cell wall of these organisms has also received considerable attention [24]. Moseley [25] has reviewed the mechanisms of the enzymic system for repairing the radiation-induced damage in DNA of the cell.

3.3. Shifts in microflora

During different phases of spoilage, fish microflora becomes diverse in nature, consisting of Gram-positive and Gram-negative organisms acquired from various sources. Pasteurizing doses of radiation reduce the total bacterial load. However, due to the differential radiation sensitivity of organisms, certain qualitative and quantitative shifts in microflora occur at low dose levels. Selective changes in bacterial groups resulting from irradiating different fish species have been extensively reported [5,6,26,27]. This selection in flora is influenced by radiation dose, type of fish, storage conditions, radiation sensitivity and initial load of bacteria.

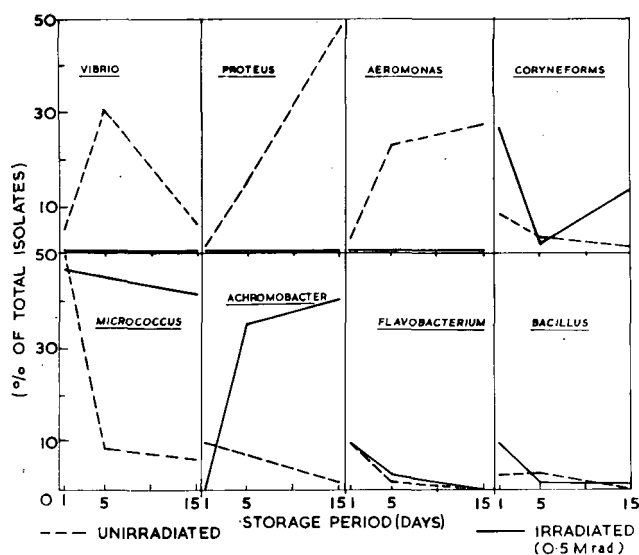


FIG. 2. Shift in microflora of Bombay duck (storage temperature 10–12°C): bacterial cultures were isolated and identified from unirradiated and irradiated Bombay duck fillets during storage at 10–12°C [5]; the percentage of microbial groups were calculated from the total number of isolates in the respective samples.

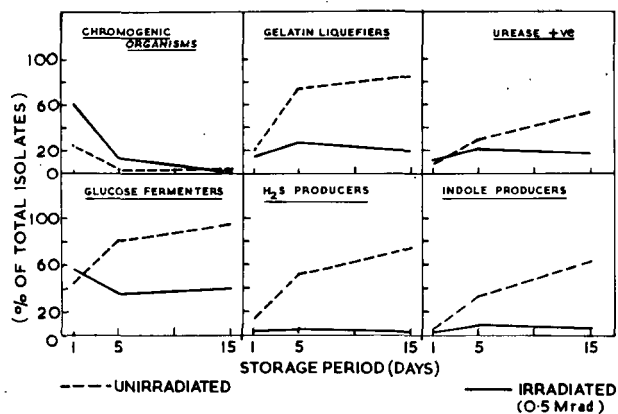


FIG. 3. Distribution pattern of the spoilage organisms in unirradiated and irradiated Bombay duck during storage at 10–12°C: bacterial cultures isolated from fresh and irradiated Bombay duck fillets (0.5 Mrad) were examined for the biochemical characteristics shown in the figure; the percentage cultures on the basis of total isolates from each group show the distributions of spoilers in irradiated and unirradiated fillets [6].

Figure 2 depicts the post-irradiation qualitative changes in microflora of Bombay duck in terms of generic distribution. In unirradiated samples, there is a marked reduction in Micrococci and Coryneforms and increase in Vibrio, Proteus and Aeromonas. On 15th day of storage, Proteus and Aeromonas are among the most predominant ones. In irradiated Bombay duck, chromogenic Micrococci are the major survivors. Non-chromogenic Micrococci and Achromobacter, however, increase rapidly and form the major groups in irradiated samples, stored for 15 days at 10–12°C.

3.4. Biochemical characteristics of micro-organisms

The selection of microbial groups, relatively inactive in spoilage reactions, forms the basis of the radiation-pasteurization process for flesh foods. This is evident from Fig. 3 which summarizes the distribution pattern of the organisms, with respect to the biochemical characteristics concerned with the spoilage potential of unirradiated and irradiated Bombay duck, during storage at 10–12°C. Prior to spoilage, the unirradiated fish included spoilers as well as non-spoilers with reference to indole, H₂S, nitrite production, urease and proteolytic activity. During further storage, however, there was a selective growth of spoilers and the flora consisted mostly of proteolytic and biochemically active organisms. In contrast, the microflora of samples immediately after irradiation was comprised mostly of chromogenic organisms and were devoid of rapid spoilers. During storage also, there was no significant increase in spoilers.

When the major radiation survivors were examined in model systems, for their spoilage potential in terms of loss in organoleptic properties and formation of chemical indices of freshness, it was observed that the Micrococci species showed their inability to produce trimethylamine from sterilized Bombay duck drip [6] or homogenates [28]. Formation of TVBN, in contrast to that of Pseudomonas, P.Vulgaris and A.hydrophila, was more suppressed in the Gram-positive organisms tested.

3.5. Radiation pasteurization of Bombay duck

The marked differences in the radiation sensitivities and biochemical properties of the predominant organisms are reflected in the keeping qualities of irradiated Bombay duck, in contrast to the unirradiated samples, similarly stored.

As already summarized in Table III conventional methods for preserving Bombay duck do not lend themselves for this fish in the fresh or 'as-is' form. In contrast, irradiation extends the storage life of this perishable fish. Radiation doses, 0.1 and 0.25 Mrad can be selected for radiation pasteurization of Bombay duck since they confer an extension in shelf life without loss in organoleptic attributes.

TABLE IV. EXTENSION IN THE SHELF LIFE OF BOMBAY-DUCK FILLETS BY RADIATION PASTEURIZATION (storage temperature: 0—2° C)

Eviscerated, cleaned, fresh Bombay-duck fillets sealed in polythene pouches were subjected to the radiation doses shown in the table; storage properties were determined on the basis of methods adopted in the authors' laboratory [4]

Treatment	Shelf life (days)	Drip loss (%)	Storage properties
Unirradiated	5—7	10—15	Characteristic putrid odours; rise in bacterial load above 10^8 /g indicates spoilage concurrent with rise in chemical indices.
0.1 Mrad	18—20	10—15	Slight putrid odours during terminal spoilage; initial lag in bacterial growth reflected in suppression of TMAN and TVBN levels.
0.25 Mrad	25—27	20	Initial irradiation odours disappear during storage; terminal spoilage indicated by stale odours; inverse relationship between rise in bacterial load to decline in organoleptic score not observed.
0.5 Mrad	35—40	25	
1.0 Mrad	45—50	25	

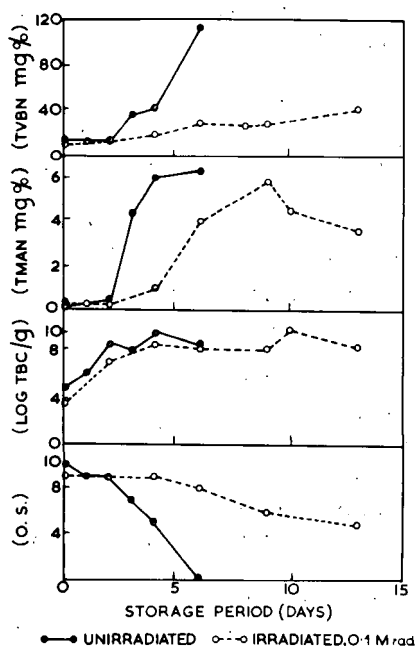


FIG. 4. Status of freshness indices in Bombay-duck fillets at 10°C; fresh Bombay duck, after evisceration and cleaning were filleted, sealed in polythene pouches and stored at 0—4°C; a radiation dose of 0.1 Mrad was used for pasteurization; samples were analysed at intervals for freshness indices as per the methods adopted in the authors' laboratory [4].

3.6. Storage properties

Table IV incorporates data on the extension in shelf life and some of the gross physical changes in terms of drip loss and irradiation odours of Bombay duck subjected to different radiation doses. It can be seen that extension in shelf life is dose-dependent and related to the storage temperature [4].

Figure 4 incorporates data on the storage properties of Bombay duck evaluated in terms of retention of freshness indices: organoleptic score (OS), total bacterial load (TBC), trimethylamine (TMAN) and total volatile basic nitrogen (TVBN).

Unirradiated Bombay duck show recognizable spoilage due to the formation of putrid odours concomitant with a precipitous fall in the organoleptic score from 10 to 5 within 7 days at 0°C. These changes are also reflected in the rise in TBC, TMAN and TVBN which reached the spoilage level.

The low irradiation dose of 0.1 Mrad extended the shelf life of Bombay duck by 17–19 days at 0°C. At the terminal spoilage, these samples developed putrid odours of low intensity which reduced the organoleptic score from 9 to 3 after 19 days at 0°C. TBC showed an initial lag followed by a rapid increase to the spoilage level of 10^8 /g. The high TBC, however, did not adversely reflect on organoleptic properties, as the samples were rated acceptable, the OS being 6–7. These findings corroborate the observations by Spinelli et al. [29]. In our laboratory, a lack of correlation between TBC and OS in irradiated samples during storage has been consistently observed [4,9].

Despite the high TBC, formation of TMAN and TVBN was suppressed considerably in irradiated samples. Low-dose irradiation of 0.1 Mrad, therefore, confers an extension in shelf life and markedly alters the storage properties of Bombay duck in terms of microbiological and chemical indices.

3.7. Spoilage units

Since bacterial spoilage or deterioration in quality, during storage by inter-molecular reactions within food constituents, is a progressive reaction, the rate of spoilage expressed in terms of units/day serves as an important parameter of storage changes. The magnitude of suppression in the spoilage rate can thus be used for defining the process treatment. Spencer and Baines [30] derived spoilage units for the organoleptic score, TMA-index and total bacterial load, using the least-square method. The rate of increase in these spoilage units was found to be a function of temperature.

Figure 5 shows the spoilage rates for unirradiated and irradiated (0.1 Mrad) Bombay duck stored at 0–2°C and 10–12°C [31]. As can be seen, the spoilage rates are higher for unirradiated Bombay duck than for irradiated samples. The inverse relationship between fall in organoleptic score and rise in TBC, TMAN and TVBN is seen from the negative slope values for the OS and positive slope values for other indices.

The spoilage rate of 0.86 calculated for the organoleptic score of unirradiated Bombay duck stored at 0°C is found to be much higher than the

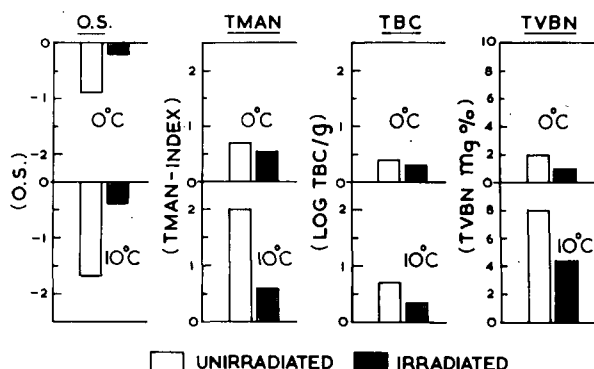


FIG. 5. Spoilage rates of irradiated and unirradiated Bombay duck (storage temperature 0–2°C and 10–12°C): spoilage rates were determined by the least-square method [30] from the storage data for unirradiated and irradiated (0.1 Mrad) Bombay-duck fillets stored at 0–2°C and 10–12°C.

rates reported by Spencer and Baines [30] for cod varieties from Hull and Aberdeen. These were found to be in the range of 0.23 – 0.3 at 0°C. Differences in spoilage rates in fish species can be expected due to variations in spoilage organisms and compositional differences within the fish species. Despite the high spoilage rate of Bombay duck, radiation-pasteurization (100 krad) augments the storage life by 18 days at 0°C.

3.8. Chemical and physical changes

As can be seen from Tables III and IV, Bombay duck provides an excellent test system for the study of textural alterations by process variables. This fish has a low water-holding capacity as observed from the extensive exudation of drip by a single freeze-thaw process or by storage at 0°C; mild heat treatment results in the disintegration of tissue. Since Bombay duck is susceptible to extensive drip losses by process treatments such as chilled storage, freezing or irradiation, it was desirable to develop pre-treatments to minimize the textural changes. Pre-dip treatments in salt solutions have been suggested to minimize drip losses and to improve the textural quality of frozen products [32–35]. Spinelli et al. [36] have employed dip treatments in solutions of sodium chloride or sodium tripolyphosphate to minimize drip losses in irradiated halibut, ocean perch and petrale sole. Gore and Kumta [7] developed optimum conditions for pre-dip treatments based on the modifying influence on physical and chemical changes in Bombay duck by irradiation.

3.9. Exudation of drip and its constituents

Figure 6 shows the marked effect on exudation of drip in Bombay-duck fillets by pre-dip treatments in solutions of either 10% sodium tripolyphosphate, 10% sodium pyrophosphate for 15 minutes or in 10% sodium chloride for 60 minutes [7].

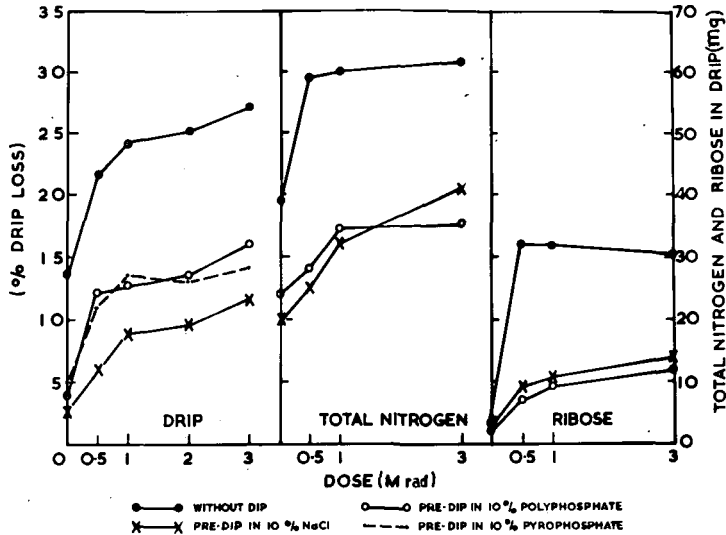


FIG. 6. Effects of pre-dip treatments on the drip and some constituents from irradiated Bombay duck: Bombay-duck fillets were treated with 10% sodium pyrophosphate and sodium tripolyphosphate solutions for 15 min and 10% sodium chloride for 60 min; untreated fillets served as control; after dip treatment, fillets were packed in polythene bags, irradiated at 0.5, 1.0, 2.0 and 3.0 Mrad at 0–2°C, stored at 10–12°C for 3 days and the drip loss measured; total nitrogen, non-protein nitrogen and ribose were determined from the drip [8].

On irradiation, the drip loss increased linearly up to 1.0 Mrad (being 20 and 24% at 0.5 and 1.0 Mrad). When higher doses of irradiation were employed, no appreciable rise in drip loss was observed. The efficiency of salt treatments in reducing the drip loss was as follows:

NaCl > sodium pyrophosphate > sodium tripolyphosphate > water

Untreated fillets which served as control showed a loss of 39 mg of total nitrogen, the protein nitrogen/non-protein nitrogen (PN/NPN) ratio being 1.16. Salt treatments reduced the loss of total nitrogen in the drip, the PN/NPN ratio being 1.72. The loss of ribose was, however, unaltered in unirradiated fillets with or without treatment.

In irradiated fillets without any pre-dip treatment, the loss of total nitrogen in the drip increased to 60 mg (0.5 Mrad) as against 39 mg in unirradiated samples. This, however, appeared to arise mainly from NPN fraction, the PN/NPN ratio being 0.9. Total nitrogen lost in the drip of irradiated fillets did not seem to be affected by increasing the radiation dose further.

Dip treatment in solutions of sodium chloride or sodium tripolyphosphate reduced the loss of total nitrogen in the drip of irradiated fillets and particularly in the NPN fraction. The ratio of PN to NPN in the drip of unirradiated samples treated with sodium chloride solution was 1.72 while in the drip of samples treated with sodium-tripolyphosphate solution it was 1.94. These ratios decreased to 1.44 and 1.40, respectively, when high doses of

TABLE V. EFFECT OF PRE-DIP IN SODIUM-TRIPOLYPHOSPHATE ON THE EXTRACTABILITY OF SALT-SOLUBLE PROTEINS IN BOMBAY-DUCK MUSCLE SUBJECTED TO DIFFERENT TREATMENTS

A solution of 5% NaCl in 0.01-M phosphate buffer, pH 7.5 and ionic strength 0.9 was used for the extraction of salt-soluble proteins [8]

Treatment	Storage	Extractable ^a proteins in 5% NaCl, pH 7.5	
		without dip (%)	with pre-dip (%) ^b
Unirradiated	without storage	75	77
Unirradiated	3 days, 0—2°C	57	71
Irradiated, 0.5 Mrad	3 days, 0—2°C	75	75
Irradiated, 3.0 Mrad	3 days, 0—2°C	35	36
Frozen at -20°C	3 days, -20°C	44	72
Steam-cooking		20	20

^a Expressed as the percentage of total nitrogen.

^b Bombay-duck fillets were dipped in 10% sodium tripolyphosphate for 15 min.

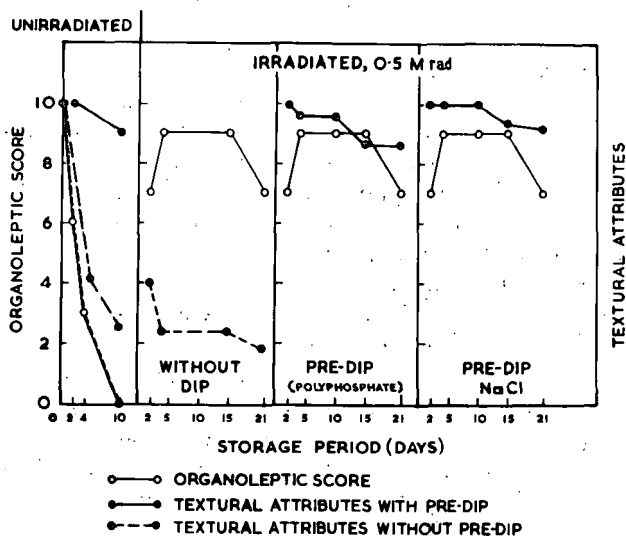


FIG. 7. The organoleptic score and textural attributes of irradiated and unirradiated Bombay-duck fillets: Bombay-duck fillets were dipped in water, 10% sodium tripolyphosphate for 15 min or 10% sodium chloride for 60 min, packed in polythene bags, irradiated with 0.25 Mrad and stored at 10—12°C; unirradiated bags served as control; the product was evaluated by organoleptic score [13] and textural quality [8].

radiation (3.0 Mrad) were employed. The leaching of ribose in drip of salt-treated irradiated samples (0.5 Mrad) was only 7–9 mg as against 32 mg in the drip of irradiated samples without dip treatment. The loss of ribose in the drip of fillets with and without treatment did not increase appreciably when higher doses were employed.

3.10. Extractability of salt-soluble proteins

Fibrillar proteins constitute approximately 55–60% of the proteins of Bombay duck. In-situ aggregation of these proteins by processing conditions which cause drip loss may be reflected in the solubility behaviour of these proteins. This was tested in a series of experiments and Table V summarizes the changes in the extractability of 5% salt-soluble proteins as influenced by various treatments [8].

The initial extractability of Bombay duck muscle proteins which is 75%, falls to 57% and 44% on storage for 3 days at 0°C and -20°C respectively. However, pre-treatment of the fillets with sodium tripolyphosphate prevents the loss in the extractability. Irradiation with 3.0 Mrad also brings about a reduction in the extractability (35%) of proteins, but this effect is not modified by sodium tripolyphosphate pre-treatment. Likewise, complete denaturation was observed in samples subjected to steam-cooking.

It is interesting to note that irradiation with 0.5 Mrad brings about drip loss, but this is not reflected in the decreased extractability of muscle proteins.

3.11. Textural stability

The in-situ modifying influence of salt-dip treatments was further investigated for reducing the textural loss of Bombay duck stored after irradiation [7]. The results have been incorporated in Fig. 7. It can be seen that unirradiated fillets became unacceptable within four days irrespective of the dip treatment prior to storage. The fillets exposed to 0.5 Mrad (dip-treated and untreated) had an irradiation odour and thus registered a lower score of 7 on the second day, but increased to 9 due to the disappearance of irradiation odours up to about 15 days. The samples were acceptable up to 21 days with a score of 7.

The fillets without pre-dip treatment showed poor textural quality with a score of 4 after irradiation due to 20% loss of drip. However, fillets dipped in solution of 10% sodium chloride for 60 minutes or in 10% sodium tripolyphosphate for 15 minutes did not show any textural damage after irradiation and thus were given a textural rating of 10 as for fresh samples. The textural rating of irradiated fillets given pre-dip treatment decreased gradually and at the end of 21 days at 10–12°C, it was 7. In contrast, fillets subjected to irradiation treatment only showed an initial low textural rating of 4 which dropped to 2 after 21 days storage.

In other studies, Gore and Kumta [7] observed that these salt-dip treatments confer textural stability to frozen Bombay duck.

The microbiological and associated biochemical studies on Bombay duck establish that bacterial spoilage can be arrested by the radiation-pasteurization process. To minimize the drip losses resulting in textural impairment and flavour losses, however, the pasteurization process should be coupled with dip treatments in salt solutions.

3.12. Radiation preservation of Bombay-duck laminates

Recently, a simple process for obtaining Bombay-duck laminates has been developed in this laboratory [37]. This consists of removing the free water from fresh Bombay duck by pressing under two wooden plates for about two minutes. The laminates, sealed in polythelene bags, exposed to 0.25 Mrad show a three-week extension in shelf life at 0°C, as observed with the fillets. By reducing the moisture content to 40%, laminates (with high rehydration and cooking properties) can be stabilized for room-temperature storage by a radiation dose of 0.25 Mrad. When the products are further air-dried (5–10%) the deterioration of the product by maggots during storage can be effectively controlled by a low-dose irradiation of 15 krad.

3.13. Radiation disinfestation of sun-dried fishery products

Commercially sun-dried products have established a good market both for inland distribution and export. However, due to high humidity and temperature storage conditions, the product is often infested with insects. Fumigation processes are ineffective in eliminating the eggs and often maggots survive as they establish themselves in the tissue. A large quantity of low-quality product, therefore, goes only as manure. Low doses of radiation in the range 15 to 25 krad were, therefore, explored for disinfesting Bombay-duck laminates. As shown in Fig. 8, irradiated laminates packed in polythene pouches did not show any damage from insects when stored for over a year.

4. SHRIMPS

Marine shrimp catches are dominated by penaeids comprising Penaeus Indicus, P. monodon, Metapenaeus affinis, M. dobsini, M. minoceros, M. brevicornis and Parapeneopsis stylifera, Metapanaeus sp. and P. stylifera which constitute the large-size and exportable category of prawns were used in our studies. Two major factors influence the keeping qualities of shrimps at 0–2°C. Development of black spots by enzymes of melanosis and autolysis occur simultaneously with putrefactive changes associated with the proliferation of psychrophiles. Radiation-processing procedures have been developed for enhancing storage stability at refrigeration temperatures of 0–2° and 10–12°C. Since sun-dried commercial shrimps occupy a major portion of the shrimps processed in India, semi-dried shrimps with high rehydration properties, colour and flavour characteristics are stabilized by the dehydro-irradiation process [10].

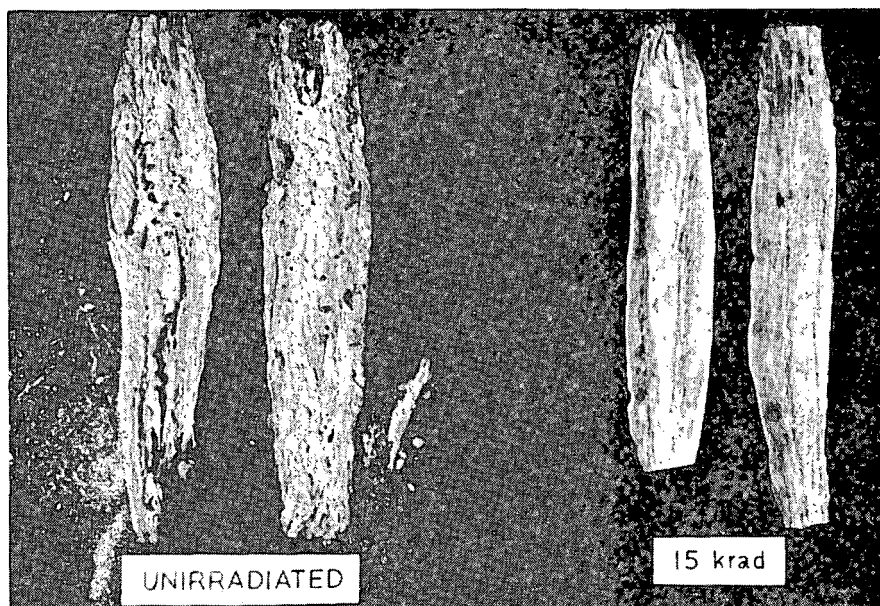


FIG. 8. Radiation disinfestation of Bombay-duck laminates: commercially available sun-dried Bombay-duck laminates were packed in polythene bags (700 gauge) and irradiated at 15 krad; unirradiated laminates served as control; samples were stored at room temperature (26–30°C) and examined for infestation.

4.1. Radiation-pasteurization process for shrimps

At radiation doses above 0.25 Mrad, the naturally occurring astaxanthine pigment is readily destroyed. Irradiation odours formed at doses of 0.5 to 1 Mrad are transitory in nature and disappear during storage [8], but the products show a lower acceptability due to loss of colour. An radiation dose of 0.15 Mrad satisfies the conditions for the optimum tolerance dose in respect of suppressing the major psychrophilic spoilage organisms and retaining the organoleptic attributes. The extension in shelf life of de-veined and peeled shrimps, sealed in polythene pouches and exposed to 0.15 Mrad, was found to be about 10–14 days and 18–20 days respectively at 10–12°C and 0–2°C. In contrast, unirradiated shrimps similarly stored, spoiled within 3–7 days [9].

Figure 9 shows the marked differences in the status of quality characteristics for unirradiated and irradiated shrimps. Irradiated samples showed a significant lag in the formation of TVBN and TMAN, in contrast to the rapid linear rise reaching spoilage levels in unirradiated samples. Temperature-dependent retardation in the quality indices is also evident from Fig. 9.

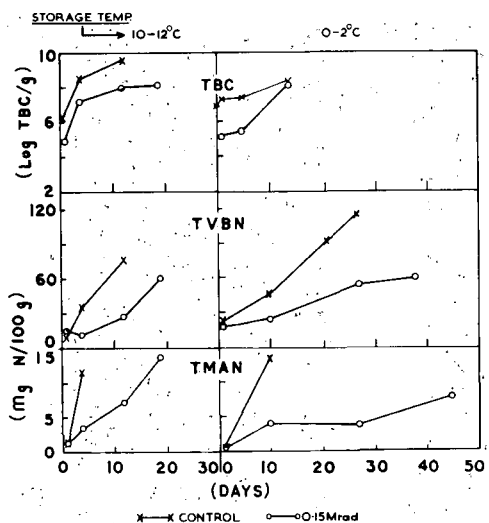


FIG. 9. Status of freshness indices of radurized fresh shrimps: peeled and de-veined fresh shrimps were sealed in polythene pouches and irradiated at 0.15 Mrad, unirradiated samples serving as control; the chemical and microbiological quality of the product was analysed as for Bombay duck [4, 9].

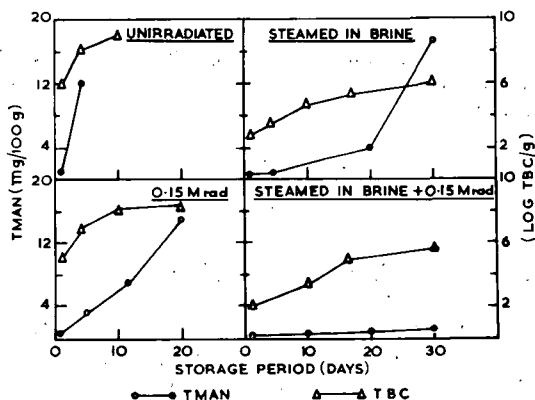


FIG. 10. TBC and TMAN of shrimps subjected to combination treatments (storage temperature 10–12°C): fresh shrimps steamed for 4 minutes, sealed in polythene pouches or cans containing brine (2.5% NaCl) and irradiated at 0.15 Mrad; unirradiated shrimps served as control; samples were stored at 10–12°C, and analysed for TBC and TMAN [9].

4.2. Combination treatments

Development of black spots due to melanosis and autolysis is a common feature in stored shrimps. In irradiated shrimps, the degree and onset of blackening was found to vary with several factors such as age, size and species of shrimps, pre-irradiation time and storage conditions. Enzyme inactivation by

a blanching treatment, consisting of steaming for 4 minutes, effectively eliminates the formation of black spots as well as thermolabile spoilage organisms. Such products are, however, not stable during storage at sub-room temperature. Aerobic Bacilli predominate leading to deterioration in quality by the development of slime and musty odours. As shown in Fig. 10, combination treatment with 0.15 Mrad was more effective in suppressing TEC. TMAN levels did not show any significant rise over 2—3 mg per cent during 30 days storage. The quality score for texture, colour, flavour and taste on a nine-point hedonic scale did not drop below 7. A blanching treatment of shrimps packed in brine helps in the retention of typical shell-fish flavours, otherwise reduced during steaming. Packaging in brine can now be substituted by blanching in 10% sodium chloride for 5 minutes at 80°C. The combination treatment of blanching followed by irradiation enhances the storage life to about 60 and 130 days, respectively at 10—12°C and 0—2°C.

4.3. Pre-cooked shrimps

The low-temperature requirement (0 to 10°C) for the combination process described above, if eliminated, would have immense potentialities for the transportation of the product at ambient temperature and for meeting the requirements of the consumer market. A process, recently developed in our laboratory [38] successfully stabilizes pre-cooked shrimps for storage at room temperature (28—32°C) for a period of 2 to 3 months. For this purpose, the initial thermal treatment is so selected as to be minimal for cooking shrimps (100°C, 5 lb/in² for 10 minutes) without much loss in texture, flavour, colour and other organoleptic attributes.

The shrimps are then sealed in cellophane polythene pouches and exposed to a radiation dose in the range of 0.15 to 0.25 Mrad. This product is devoid of bacterial decomposition as well as blackening and retains the characteristics of the product. Pre-cooked shrimps stored at room temperature without any radiation treatment spoil within 3 days due to the predominance of aerobic spore-bearing bacilli belonging to B. megaterium. The process has thus unique advantages of low dose, minimum pre-treatments and room-temperature stability of the product. Further studies are in progress to establish the physiological conditions unfavourable for the growth of Cl. botulinum. One approach being tested is thermal inactivation at a low pH followed by neutralization of the product.

4.4. Dehydro-irradiation process

The advantages of stabilizing partially dried seafoods by low-dose irradiation, referred to as 'dehydro-irradiation' process has been reported from this laboratory [2,10,39]. Such products successfully substitute for sun-dried commercial samples in terms of rehydration properties, taste, texture, appearance and flavour. The process integrates:

- (i) The inactivation of enzymes by blanching at 80°C for 5 minutes in 10% sodium chloride.

TABLE VI. STORAGE LIFE OF SHRIMPS WITH DIFFERENT MOISTURE CONTENTS^a

	Rehydration ^b	Storage life (days)		Spoilage characteristics ^c
		Storage temperature 10—12° C	25—30° C	
Fresh, 80% moisture		3	few hours	Typical putrid odours and high bacterial load
50% moisture	2.0	10—12	4—5	Heavy mould growth and ammoniacal odours, high bacterial load
40% moisture	1.4	10—12	4—5	Heavy mould growth and ammoniacal odours, high bacterial load
30% moisture	1.2	15—20	7—10	Heavy mould growth, ammoniacal odours
20% moisture	0.9	60—65	30—35	Mould growth and ammoniacal odours

^a Fresh prawns were packed in polythene bags and stored at 10—12° C; fresh shrimps were cooked and dehydrated to different moisture levels, packed in polythene bags and stored at 28—30° C.

^b Rehydration was done in distilled water for 3 hours; the rehydration value is expressed as the water content per gram of dry material.

^c Spoilage of fresh shrimps is characterized by putrid odours while semi-dried shrimps show a quick onset of mould.

(ii) Partial control of mould by sorbic acid (0.5%) dip treatment for 1 hour.

(iii) Semi-drying to 40% moisture.

(iv) Sealing in flexible packaging containers.

(v) Irradiation, 0.25 Mrad.

Each of the process variables has been carefully selected in the sequence indicated above, so that each treatment is complementary to the other. Some of the data concerning the benefits of each of the above treatments will amplify this point.

Table VI shows the spoilage pattern and rehydration properties of shrimps varying in moisture content. The storage life is an inverse function of the water activity of bacteria; therefore, products containing 40—50% moisture spoil rapidly. As these products, compared to products with lower moisture content, had better rehydration properties, the final moisture level of the product was held at 40%.

When the temperature of inactivation of enzymes was changed from steaming for 10 minutes to heat treatments at 60° and 80° C, there was an improvement in the rehydration properties of the semi-dried (40% moisture) products (see Fig. 11). As heat treatment at 60° C for 5 minutes was inadequate for inactivation of enzymes of melanosis, heat treatment at 80° C for 5 minutes was adopted.

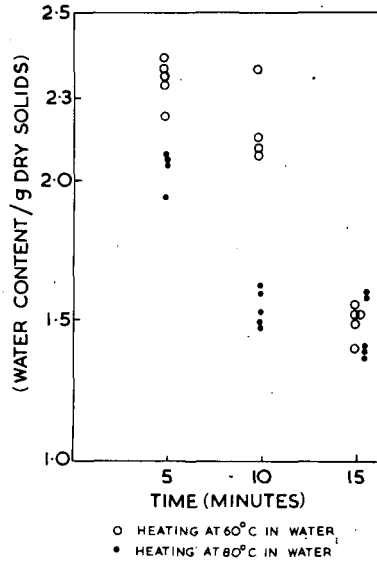


FIG. 11. Rehydration properties of semi-dried shrimps: fresh peeled and de-veined shrimps were blanched in water at 80°C or 60°C for 5, 10 and 15 minutes and dehydrated to a 40% moisture level; semi-dried shrimps were rehydrated for 3 hours in distilled water; the rehydration value is expressed as the water absorbed per gram of dry solid [10].

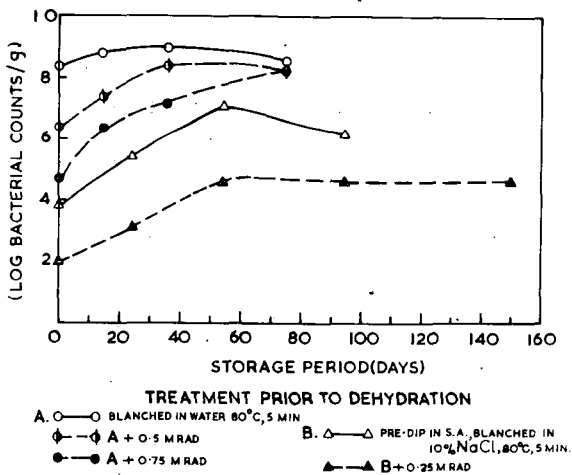


FIG. 12. Facterial load of semi-dried shrimps: fresh peeled and de-veined shrimps were blanched, dehydrated to 40% moisture level, packed in polythene bags (700 gauge) and exposed to 0.25 Mrad; total viable bacterial load was determined at intervals during storage at room temperature (27–30°C) [10].

Since low doses of radiation do not eliminate mould, dip treatment in sorbic acid (0.5%, 60 minutes, pH 6.8) was given after a blanching treatment at 80°C for 5 minutes in 10% sodium chloride. The sorbic acid content of semi-dried shrimps (25 mg%) is within permissible limits and the product containing 3.25 g% sodium chloride does not give an excessive salty taste. However, dip treatment alone did not control mould beyond 30 days. An extension in shelf life up to 150 days was obtained only by irradiation at 0.25 Mrad. The progressive reduction in bacterial load in semi-dried shrimps and the extension in shelf life by each of the above treatments are shown in Figs 12 and 13 respectively. The synergistic effect of sorbic acid-coated cellophane wrappers and low-dose irradiation has been reported for dairy products from this laboratory [40,41].

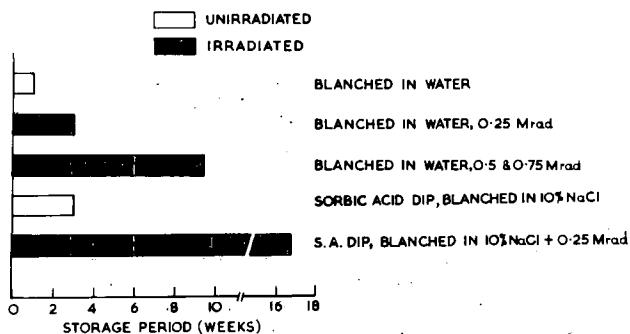


FIG. 13. Extension in storage life by combination treatments: fresh shrimps, subjected to the treatments shown in the figure, were evaluated for the onset of mould and organoleptic characteristics during storage at room temperature (26–30°C) [10].

TABLE VII. MOISTURE RETENTION OF SEMI-DRIED SHRIMPS DURING STORAGE IN DIFFERENT PACKAGING MATERIALS^a

		Moisture content (%)	
		90 days	180 days
(1)	Laminated cellophane pouches	36.4	34.5
(2)	Aluminium-paper-polythene pouches	36.0	35.0
(3)	Laminated cellophane, MST	30.5	25.4
(4)	Laminated paper pouches	30.0	26.3
(5)	Polythene bags, 700 gauge	36.7	35.0
(6)	White-opaque trayopol	30.8	27.4
(7)	Polythene bags, 100 gauge	30.2	26.3

^a Shrimps dipped in 0.5% sorbic acid for 60 minutes and blanched in 10% sodium chloride (80°C, 5 minutes) were dehydrated at 55–60°C to a 40% moisture level; the semi-dried shrimps were then packed in packaging materials and irradiated with 0.25 Mrad; the moisture content was determined on 90 and 180 days storage; packaging materials described under (1), (2) and (5) appear to retain moisture on extended storage.

4.5. Packaging of semi-dried shrimps

Loss of moisture of semi-dried shrimps during storage due to evaporation reduces textural and flavour characteristics. Hence, the moisture-retention properties of several commercially available flexible packaging materials were examined: As shown in Table VII, laminated cellophane pouches, film polythene pouches, and polythene bags (700 gauge) were most effective in retaining moisture during prolonged storage. In contrast, shrimps packed in polythene bags (100 gauge), laminated cellophane MST, laminated paper pouches and opaque tryopol MST showed a moisture loss of 10–14% and developed a hard texture during a storage period of 3 and 6 months.

TABLE VIII. RADIATION PRESERVATION OF TROPICAL SHRIMPS^a

Process	Storage temperature (°C)	Storage properties	Shelf life (weeks)
<u>Treatments without irradiation</u>			
Conventional chilling	0–2	Bacterial spoilage, melanosis or blackening	1
Blanching (100°C, 4 minutes)	10–12	Arrests melanosis but susceptible to bacterial spoilage	3
Semi-dried (40% moisture)	28–34	Rapid onset of spoilage by mould and bacteria	1
Semi-dried (40% moisture, sorbic acid dipped)	28–34	Bacterial spoilage delayed	3
Cooked (100°C, 5 lb/in ² , 100 minutes)	28–34	Enzymes inactivated, rapid bacterial spoilage	3 days
<u>Radiation pasteurization (dose: 0.15 to 0.25 Mrad)</u>			
Fresh	10–12	Controls spoilage flora but not melanosis	2
	0–2	Controls spoilage flora but not melanosis	3
Blanched (100°C, 4 minutes)	10–12	Controls bacterial spoilage and melanosis	8
	0–2	Controls bacterial spoilage and melanosis	12
Cooked (100°C, 5 lb/in ² , 100 minutes)	28–34	Controls bacterial spoilage and melanosis	8
<u>Dehydro-irradiation (dose: 0.25 Mrad)</u>			
Semi-dried (40% moisture)	28–34	Shelf stability limited	3
Semi-dried (40% moisture, sorbic acid dipped)	28–34	Extended shelf stability	18

^a De-veined and peeled fresh shrimps were subjected to different treatments as required for the process indicated in the text.

Among the packaging materials tested, laminated cellophane pouches showed a decreased oxygen permeability and are thus suitable for vacuum-packing semi-dried shrimps. Materials such as aluminium laminates, polyvinylidene chloride-based films (Saran), coated polyesters (mylar) and nylon-11, which are effective gas and water-vapour barriers, are being tested for their suitability. These studies [42] show that oxidative deterioration and the growth of micro-organisms could be further inhibited by the anaerobic packing of the dehydro-irradiated product.

Table VIII summarizes the radiation-processing procedures and storage characteristics of fresh, blanched, cooked and semi-dried shrimps.

5. POMFRETS

Hitherto, pomfrets have not been processed either by conventional methods of canning or drying. In recent years, during the glut season, a major portion of pomfret catches are being quick-frozen and stored in frozen storage for marketing subsequently during the slack season. This fish, however, is most susceptible to oxidative changes during frozen storage. These changes are manifested in terms of the development of yellow discolouration. Glazing methods have been described for minimizing dehydration and textural losses of frozen pomfret. Radiation-pasteurization processes coupled with anaerobic packaging effectively arrest radiation-induced oxidative changes and offer considerable scope for preserving pomfrets in an 'as-is' or fresh form.

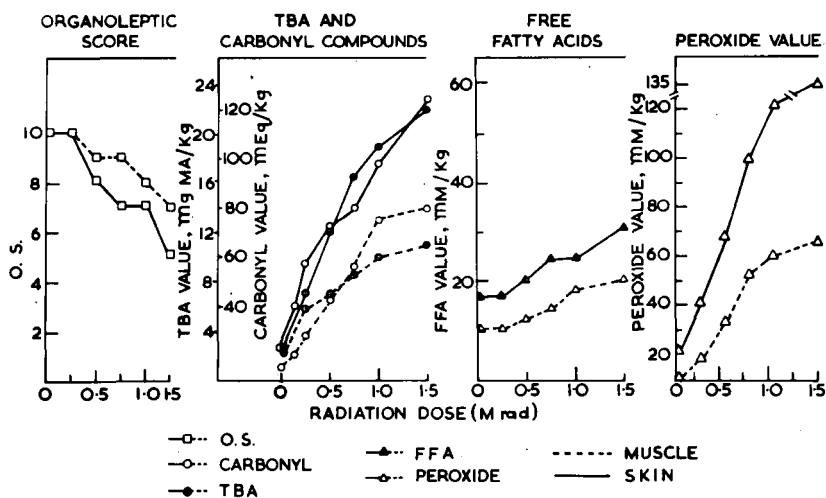


FIG. 14. Radiation sensitivity of skin and muscle of white pomfrets: skin as well as muscle samples of pomfret were exposed to doses in the range of 0.25 to 1.5 Mrad; unirradiated samples served as control; the samples were examined immediately for oxidative changes in terms of TBA [44], FFA [45], carbonyl [46] and peroxide [47] values; the skin is more susceptible to radiation-induced oxidative changes than the muscle.

5.1. Radiation sensitivity of the skin and muscle of white pomfret

The skin and muscle of pomfret exhibit marked differences in their susceptibility to radiation-induced oxidative changes [43]. Figure 14 shows the dose-dependent changes in sensory attributes in relation to oxidative deterioration in skin and muscle. A sharp rise in thiobarbituric acid [44], free fatty acids [45], carbonyl [46] and peroxide values [47] with increase in radiation dose results in the development of a 'cod-liver oil-like' or rancid type of odour. In muscle, there was a gradual increase in oxidative changes, but the irradiation odours encountered at 1.0 Mrad were not of the rancid type. These could be described as a 'burnt-feather type'. These differences can be attributed to a higher content in the skin of total lipids, triglycerides and unsaturated fatty acids.

5.2. Influence of physical pre-treatments on oxidative changes in pomfret skin

Because of the marked radiation sensitivity of the skin, the influence of physical treatments like vacuum packing, blanching and freezing was examined for radiation-induced oxidative changes with a view to developing combination methods. Samples which did not receive any treatment served as control.

As shown in Table IX, the first significant change in the OS of aerobically irradiated (control) skin occurred at 0.5 Mrad, due to the development of an undesirable rancid type of odour [43]. Samples irradiated under vacuum did not develop such off-odours at any dose level up to 1.5 Mrad and retained a high OS. Blanching or freezing prior to irradiation showed an intermediate effect on OS. In these samples a significant rancid type of odour could be detected at 1 Mrad.

TABLE IX. EFFECT OF PRE-TREATMENTS ON THE ORGANOLEPTIC SCORE OF IRRADIATED POMFRET SKIN ^a

Radiation dose (Mrad)	Air-packed samples	Pre-treatments		
		Vacuum packing	Freezing	Blanching
(Organoleptic score)				
Unirradiated	10	10	10	10
0.25	10	10	10	10
0.50	8	10	10	10
0.75	7	9	9	9
1.00	7	9	7	7
1.50	5-6	9	6	7

^a Samples packed under aerobic and anaerobic conditions as well as pre-blanching and frozen samples were irradiated at various dose levels, ranging from 0.25 to 1.5 Mrad; within 24 hours of irradiation, samples were assessed for their organoleptic quality in terms of colour and odour.

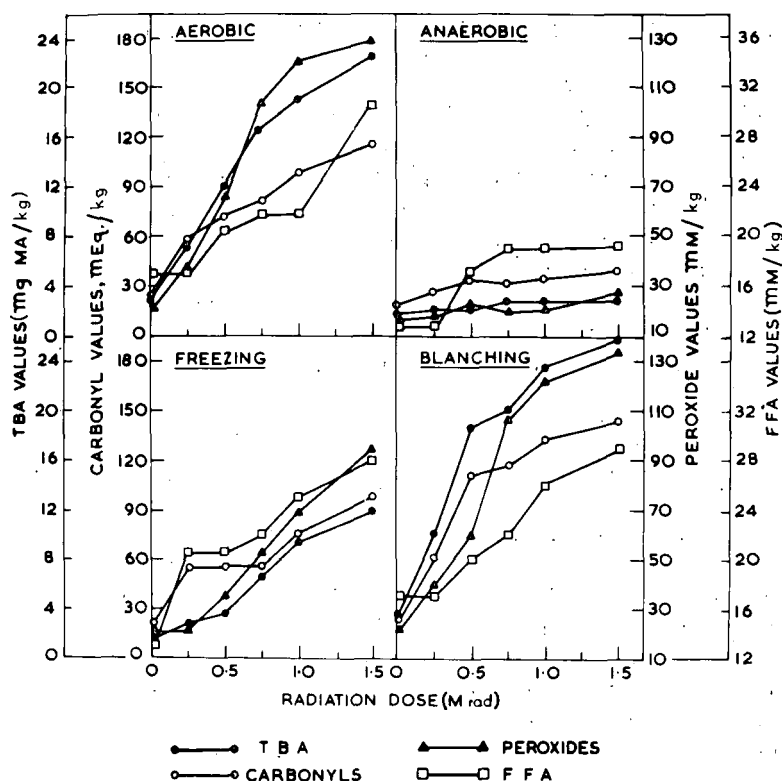


FIG. 15. Radiation-induced oxidative changes in pomfret skin: skin samples subjected to the physical treatments of vacuum packing (repeated flushing with nitrogen gas and evacuation), freezing (-20°C) and blanching (100°C , 20 minutes) were irradiated at doses ranging from 0.25 to 1.5 Mrad; for corresponding controls, the samples which did not receive these pre-treatments were used; all samples were assessed for oxidative changes as described for Fig. 14.

As shown in Fig. 15, a rise in the TBA, FFA, peroxide and carbonyl values of the skin was suppressed in vacuum-packed irradiated samples, more effectively than by other treatments studied. In blanched samples a sharp rise in TBA and carbonyl values was noticed. This, however, did not reduce the OS of the samples up to 0.75 Mrad. For example, no rancid odours were noticed although TBA, and carbonyl values were 20 mg and 118 MeQ respectively for blanched samples irradiated at 0.75 Mrad. Unblanched skin irradiated at 0.5 Mrad showed development of these odours even when the TBA and carbonyl values were 12 mg and 72 MeQ respectively. These differences may be attributed to the nature of the volatile products formed.

5.3. Suppression of yellowing by vacuum packing

TBA reactants (TBRS) formed; especially malonaldehyde, have been implicated in the formation of carbonylamine-type reactions leading to a yellow

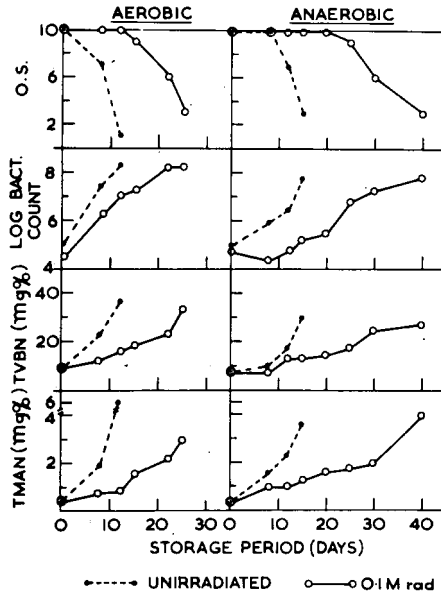


FIG. 16. Biochemical and microbial changes in irradiated white pomfret: pomfret fillets were irradiated at 0.1 Mrad under vacuum or aerobic conditions and stored at 0–2°C; unirradiated samples served as control; at regular intervals samples were examined for storage properties as for Fig. 4.

to brown colouration [48]. Pomfret fillets exposed to 0.3 and 0.5 Mrad under aerobic conditions develop yellow discolouration by the 20th and 10th day of storage respectively. The formation of TBRS by lipid oxidation involves the participation of molecular oxygen to form hydroperoxide, which being unstable, decomposes further to give a great variety of carbonyl compounds. Therefore, the effects of irradiation under anaerobic conditions on the development of yellowing were tested.

Quantification of yellowing was made by extracting the yellow pigment [49] with an acetone, HCl, methanol mixture (100 : 4 : 0.1). Figure 16 shows data on the spectral characteristics of the yellow pigment formed under aerobic conditions. No yellow pigment was formed during storage in samples irradiated and stored under anaerobic conditions.

5.4. Storage properties of irradiated pomfret fillets

Figure 16 shows data of samples exposed to 0.1 Mrad. Under aerobic conditions, there was a rapid rise in the initial TMAN, TVBN and TBC values to 3 mg, 29.5 mg and 1.9×10^8 /g respectively on the 25th day of storage, concurrent with spoilage. Terminal spoilage pattern of this sample was marked by a putrid type of odour.

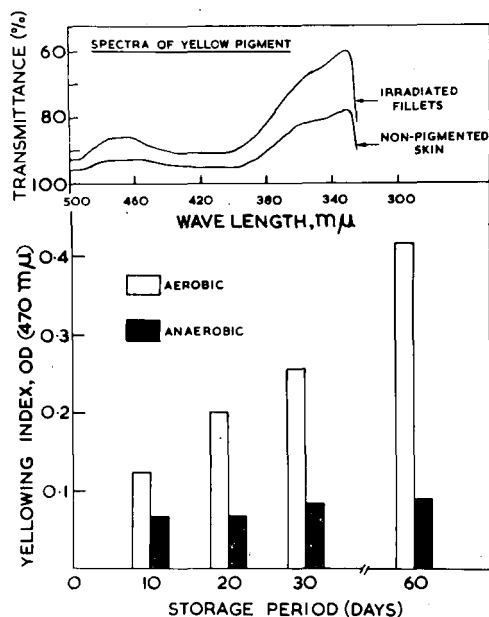


FIG. 17. Suppression of yellow pigment in anaerobically irradiated pomfret: pomfret fillets were irradiated under vacuum or aerobic conditions, at 0.1, 0.3 and 0.5 Mrad and subsequently stored at 0–2°C; yellow pigment developed in the fillets was extracted with an acetone, HCl, methanol mixture (100:4:0.1) and examined for the spectral characteristics; quantitation of yellow colour formed in the samples was made in terms of absorption at 470 mμ, of the extracted pigment.

Exposure of samples to 0.1 Mrad under vacuum showed a lag in the rise of the TMAN, TVBN and TBC levels up to the 15th day of storage. This was, however, succeeded by significant increases in these levels (see Fig. 17) until the 40th day when the spoilage was apparent. Terminal spoilage of the sample was not marked by putrid odours as in the corresponding aerobically packed sample. The unirradiated samples packed aerobically or anaerobically showed a progressive decline in quality characteristics as shown in Fig. 17. Thus, the radiation-pasteurization processing of white pomfrets essentially integrates the vacuum packaging of fillets, irradiation with 0.1 Mrad and storage at 0–2°C. This combination treatment extends the storage life for 35 days.

5.5. Transportation studies

Because of inadequate refrigeration facilities during the transport and retailing of fish, the marketable quality of fish is often reduced and has very low shelf life. Since, in irradiated fish, the initial total bacterial load is low and spoilage activity of Gram-negative organisms is reduced, irradiated fish, suitably packed in ice containers, should be more suited for inland transportation. As the spoilage rates for irradiated fish are also temperature-dependent and several

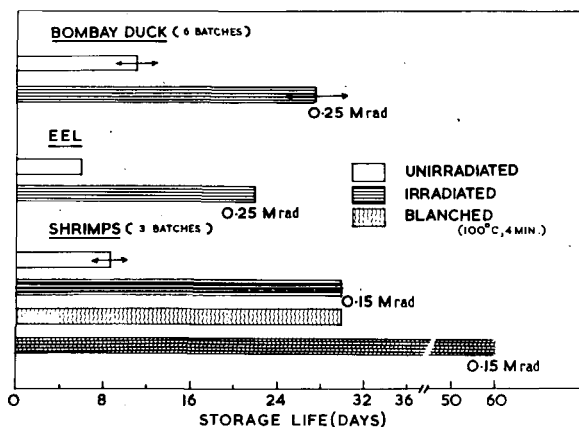


FIG. 18. Transportation studies of irradiated fishery products (received from C.I.F.T. Veraval and Bombay): different batches of fresh Bombay duck; eel and shrimps transported from Veraval in ice-boxes, packed in polythene bags (700 gauge); shrimps prior to sealing were subjected to blanching at 100°C for 4 minutes; Bombay-duck and eel fillets were exposed to 0.25 Mrad; raw and blanched shrimps (100°C, 4 minutes) were irradiated at 0.15 Mrad, unirradiated samples served as controls; the shelf life of these samples stored at 0–2°C was determined by organoleptic acceptability [4,9].

post-irradiation storage factors could influence quality characteristics, it is necessary to maintain strict control over temperature fluctuations.

Transportation studies of irradiated fish are being carried out with the active collaboration of the Central Institute of Fisheries Technology, Veraval and Bombay sub-stations, and with the Department of Food Technology and Bioengineering, Jadhavpur University, Calcutta.

During 1968–69, eight consignments of iced Bombay duck, shrimps and eel, each of about 20–25 kg were transported by train over a distance of about 1599 km from Veraval. The samples received after a pre-irradiation period varying from 1–4 days were processed only if the quality was considered acceptable. After sealing in polythene pouches, the products were exposed to 0.25 Mrad and stored at 0–2°C for evaluation. The irradiated batches were also transported in ice containers to Veraval.

Figure 18 shows the extension in shelf life for the irradiated fish. These results demonstrate that pre-irradiation storage of 4–5 days did not significantly reduce the marketable quality of irradiated Bombay duck, shrimps and eel. Ampola and Ronsivalli [50] reported that loss in marketable quality was affected only after pre-irradiation storage of 13 days at 0–2°C.

By careful control of pre- and post-irradiation storage conditions, radiation processing can be expected to result in considerable savings within the fishery trade by centralizing filleting operations as well as reducing wastage and distribution costs. Irradiated fish fillets with their increased shelf-life would allow regular and less frequent deliveries to be made to retail shops and offer significant prospects, especially for transportation from coastal regions to consumer centres.

ACKNOWLEDGEMENTS

The authors' thanks are due to their colleagues in the Division of Biochemistry and Food Technology who have shared in the experimental work reported here.

The co-operation extended in the transportation studies by Mr. R. Venkatraman, Mr. P. V. Kamasastri, Central Institute of Fisheries Technology, Veraval and Bombay sub-stations and Professor A. N. Bose, Department of Food Technology and Bioengineering, Jadhavpur University, Calcutta, is gratefully acknowledged.

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USE OF LOW DOSES OF GAMMA RADIATION FOR THE PRESERVATION OF WHITE FISH FILLETS

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Abstract

USE OF LOW DOSES OF GAMMA RADIATION FOR THE PRESERVATION OF WHITE FISH FILLETS.

Hake fillets packed in plastic bags, permeable and impermeable to gas, in a normal atmosphere and under vacuum were irradiated at 100, 150 and 200 krad with subsequent storage at refrigeration temperatures (1°C). Periodic biochemical checks were carried out on the level of decomposition and sensory assessments made on the quality of raw and of cooked fillets, using untrained tasting panels consisting of 20 and 10 judges respectively. The results are discussed and the conclusions given.

1. INTRODUCTION

Among foodstuffs of animal origin marine products, and more particularly white fish, possess exceptional economic importance for Spain in view of the volume of the catch, amounting in the particular case of hake to 73 014 tons during 1968 and equivalent in monetary terms to 2925-million pesetas.

However, the perishable nature of this foodstuff gives rise to numerous transport and preservation problems, which have not been completely solved by conventional preservation methods. As a result, distribution to the consumer is irregular and many rural areas are inadequately supplied with fish, which moreover generally reaches the consumer in a far from satisfactory hygienic state.

The use of low doses of gamma radiation in conjunction with refrigeration may resolve these marketing difficulties by increasing by up to three times the keeping period of fish in the fresh state.

The Nuclear Energy Board, which is closely concerned with this problem, is at present preparing a program for the irradiation preservation of white fish fillets and has undertaken, in conjunction with the International Atomic Energy Agency, Research Contract No. 731/RB which covers basically the following fields:

- (a) Determination of the optimal radiation dose capable of extending the storage life to meet commercial requirements.
- (b) Determination of the effect of different irradiation conditions such as ambient atmosphere and packaging materials.

A bibliography of relevant published information has been included.

2. EXPERIMENTAL PART

2.1. Raw Material

Fillets of hake (*Merluccius merluccius*) weighing approximately 100 g were used, obtained from headed and eviscerated fish from the catch of trawlers fishing off the north-west coast of Spain. The period elapsing between the catching of the fish and subsequent irradiation varied between 48 and 60 hours.

2.2. Packaging

The fillets prepared in this way were enclosed in polythene and Rilsan (French nylon) bags, respectively permeable and impermeable to gas, which had been previously sterilized by irradiation, and the packages were then hermetically sealed in a normal atmosphere or under vacuum.

2.3. Irradiation

The packaged material was divided into batches, approximately 15% of which were retained as a non-irradiated control and 10% were frozen for use as a control in the sensory tests in the cooked state.

Irradiation was effected by introducing the packaged material, surrounded with ice, into the Nuclear Energy Board's "NAYADE" ^{60}Co gamma-irradiation unit, using dose levels of 100, 150 and 200 krad.

2.4. Storage

The control and irradiated batches were stored during the experiments at refrigeration temperatures (1°C , 0.5°C):

2.5. Determination of the optimal dose

In order to establish for the fish species under examination the optimal radiation dose capable of effecting an extension of the storage life worth while from the commercial and hygienic points of view, while retaining, under optimal conditions, satisfactory organoleptic characteristics, biochemical checks of the level of decomposition and assessments of the sensory characteristics were carried out weekly from the date of irradiation.

2.5.1. Biochemical checks on the level of decomposition

To detect the first symptoms of decomposition the following determinations were carried out:

- I. Total volatile bases (TVB), using Okolov's method;
- II. Trimethylamine (TMA), using Dyer's method.

2.5.2. Sensory analysis

Using a 5-point hedonic rating scale (see Rating Sheet 1, Appendix I) and an untrained panel consisting of 20 judges drawn from personnel on the strength of the laboratory, the sensory characteristics of appearance, odour and texture of the fillets were assessed in the raw state.

In order to assess the taste of the cooked samples, a second panel was set up consisting of 10 judges selected from the previous one as having displayed greater discrimination in evaluating the raw fish samples.

The control and irradiated samples were presented to the judges under code numbers and the latter recorded individually their scores as shown in Rating Sheets 2 and 3 (see Appendices II and III respectively).

The samples used for evaluating the cooked product were prepared in the following way: The frozen controls were exposed to a steam flow for 20 minutes without previous defreezing and the irradiated samples were immersed in their packages in a bath of boiling water for 10 minutes.

In order to establish a quality criterion, all samples failing to obtain a mean hedonic-scale rating of 3 for the parameters under investigation were considered to have reached the end of their storage life. Any sample failing to score 2.8 on any individual parameter was likewise discarded.

To determine whether the mean values for each of the parameters in the sensory analysis, established by a small population such as that of our panels, could be regarded as significant in relation to large consumer groups, the results were subjected to statistical treatment using the Student t-distribution and adopting a 90% probability.

2.6. Effect of different irradiation conditions

For an assessment of the possible beneficial effects of different irradiation atmospheres and packaging materials, a preliminary test was carried out in which hake fillets packed in Rilsan bags (impermeable to gas) were irradiated with 100 krad in a normal atmosphere and under vacuum, and stored at identical temperatures.

2.6.1. Biochemical checks of decomposition

Similar checks were carried out as for the optimal dose determination.

TABLE I. BIOCHEMICAL TESTS

Week	TVB (mg N%)				TMA (mg N%)			
	Control	100 krad	150 krad	200 krad	Control	100 krad	150 krad	200 krad
0	15.4	24.4	22.5	19.6	—	—	—	—
1	36.4	26.6	23.8	26.6	6.40	1.20	0.96	0.56
2	56.0	29.4	26.6	22.4	8.40	2.40	0.60	0.60
3	—	42.0	22.4	28.0	—	2.50	1.60	0.60
4	—	61.6	39.2	37.8	—	6.00	1.16	1.00

TABLE II. SUMMARY OF SENSORY AND BIOCHEMICAL INDICES IN RESPECT OF IRRADIATED FISH AS A FUNCTION OF IRRADIATION DOSE AND STORAGE TIME (SENSORY EVALUATION RELATES TO FISH IN RAW STATE)

Irradiation dose (krads)	Storage time (weeks)	Mean sensory scores ^a			TVB (mg N%)	TMA (mg N%)
		Appearance	Odour	Texture		
100	0	—	—	—	24.4	—
	1	4.0	3.9	3.4	26.6	1.2
	2	3.3	3.2	3.1	29.4	2.4
	3	3.4	3.0	3.1	42.0	2.5
	4	2.0	2.5	2.1	61.6	6.0
150	0	—	—	—	22.5	—
	1	3.4	3.6	3.4	23.8	0.96
	2	3.8	3.7	3.7	26.6	0.60
	3	3.2	3.5	3.2	22.4	1.60
	4	3.7	3.0	3.3	39.2	1.16
200	0	—	—	—	19.6	—
	1	3.5	3.7	3.6	26.6	0.56
	2	3.6	3.5	3.4	22.4	0.60
	3	3.5	2.9	2.9	28.0	0.60
	4	3.5	2.7	3.0	37.8	1.00
0	0	—	—	—	15.4	—
	1	3.6	3.2	2.8	36.4	6.40
	2	2.1	1.4	1.9	56.0	8.40
	3	—	—	—	—	—
	4	—	—	—	—	—

^a The limited storage life is defined as the point at which the mean value for any parameter falls below 2.8.

2.6.2. Sensory analysis

This was carried out under the same conditions as for the previous experiment except that the odour was taken as the fundamental parameter, since this sensory characteristic reflects more faithfully the quality of the fish at any given moment.

The tests were carried out in the raw state on the 1st, 7th, 11th and 13th days after irradiation; each panel judge carried out duplicate evaluations.

3. RESULTS AND DISCUSSION

Table I gives the results obtained from biochemical checks over a four-week storage period of the level of decomposition in the control samples and those irradiated at different doses.

Table II shows the mean sensory assessments of the control samples and of those irradiated in the raw state, as a function of irradiation dose and storage time.

Table III gives the results obtained for the parameters assessed in the cooked state.

The values obtained in biochemical checks in respect of total volatile bases (TVB) reveal that the non-irradiated samples exceeded the figure regarded as acceptable (30 mg N%) during the first week, whereas the values obtained for the irradiated samples remained within the permissible range up to the second week irrespective of the dose used. The 100-krad sample exceeded this limit in the third week and those irradiated with 150 and 200 krads only in the fourth week.

The trimethylamine (TMA) content in the controls exceeded the maximum permissible level (1.5 mg N%) during the first week. The sample irradiated with 100 krads exhibited several values in excess of this limit in the second week. The samples irradiated with 150 and 200 krads gave figures within the acceptable limit, even in the fourth week of storage.

In the sensory evaluation of the raw fish (Table II) the control samples were rejected in the second week of storage, the 100-krad sample was rejected by the panel in the fourth week and the 150-krad sample remained acceptable throughout the four-week period. This agreed with the biochemical indices for the latter sample. The 200-krad sample was rejected in the fourth week for failing to reach the minimum quality standard (2.8) in regard to odour, although the biochemical check still gave acceptable results; this may have been due to the occurrence of foreign odours induced by irradiation at this dose level.

In the sensory evaluation of the cooked fish (Table III) in which samples frozen without irradiation were used as a control, the panel found no differences between the control and irradiated samples throughout the first two weeks. In the third week the control sample was eliminated for failing to obtain the minimum score in regard to texture; nevertheless, on the grounds

TABLE III. SUMMARY OF SENSORY AND BIOCHEMICAL INDICES IN RESPECT OF IRRADIATED FISH AS A FUNCTION OF IRRADIATION DOSE AND STORAGE TIME (SENSORY EVALUATION RELATES TO FISH IN THE COOKED STATE)

Irradiation dose (krads)	Storage time (weeks)	Mean sensory scores ^a (5-point hedonic scale)				TVB (mg N%)	TMA (mg N%)
		Appearance	Odour	Texture	Taste		
100	0	—	—	—	—	24.4	—
	1	3.8	3.6	3.7	3.8	26.6	1.2
	2	3.6	3.7	3.7	3.4	29.4	2.4
	3	3.6	3.6	3.4	3.5	42.0	2.5
	4	b	b	b	b	61.6	6.0
150	0	—	—	—	—	22.5	—
	1	3.9	3.6	3.7	3.2	23.8	0.96
	2	3.6	3.8	3.7	3.7	26.6	0.60
	3	3.7	3.9	3.5	3.4	22.4	1.60
	4	3.7	2.8	3.7	3.2	39.2	1.16
200	0	—	—	—	—	19.6	—
	1	3.7	3.6	3.8	3.7	26.6	0.56
	2	3.5	3.6	3.7	3.3	22.4	0.60
	3	3.5	3.6	3.4	3.1	28.0	0.60
	4	b)	b)	b)	b)	37.8	1.00
0 (Frozen sample)	0	—	—	—	—	—	—
	1	3.6	3.4	3.9	3.4	—	—
	2	3.2	3.5	3.2	3.7	—	—
	3	2.9	3.6	2.8	3.1	—	—
	4	2.6	2.7	2.5	2.3	—	—

^a The limited storage life is defined as the point at which the mean value for any parameter falls below 2.8.

^b No panel ratings owing to rejection of samples in the raw state.

that this was not a highly significant parameter the sample was again submitted for assessment in the following week at which stage it was rejected in respect of all parameters. All the irradiated samples obtained, in regard to all parameters, values above the minimum quality figure up to the third week. In the fourth week the 100- and 200-krad samples were not submitted for assessment as they had been previously eliminated by the panel in the raw state; the 150-krad sample, on the other hand, obtained an acceptable score although the odour rating was at the lowest permissible limit.

The result of the tests on the use of Rilsan (nylon) as a packaging material in a normal atmosphere and under vacuum, using a 100-krad irradiation dose, are given in Table IV.

TABLE IV. SUMMARY OF SENSORY AND BIOCHEMICAL INDICES OF HAKE FILLETS, PACKAGED IN RILSAN BAGS (IMPERMEABLE TO GAS) AS A FUNCTION OF PACKAGING TEMPERATURE AND STORAGE TIME

Irradiation dose: 100 krad

Packaging atmosphere	Storage time (days)	Mean odour scores	TVB (mg N%)	TMA (mg N%)
Control, normal atmosphere	1	4.2	22.4	0.96
	7	2.3	47.6	9.00
	11	—	—	—
	13	—	—	—
Irradiated, normal atmosphere	1	3.9	19.6	0.12
	7	4.1	20.2	0.20
	11	3.2	26.8	1.00
	13	3.3	27.8	1.20
Control, under vacuum	1	3.8	23.1	1.00
	7	4.2	30.8	8.80
	11	2.7	57.4	18.00
	13	—	—	—
Irradiated, under vacuum	1	3.3	21.0	0.14
	7	3.8	19.6	0.18
	11	2.9	28.5	0.28
	13	2.8	29.6	0.56

On the seventh day the control samples in a normal atmosphere recorded some unacceptable biochemical and sensory indices; this did not occur with the control samples under vacuum, which were still acceptable at this stage but were then rejected on the 11th day. The irradiated samples, whether in a normal atmosphere or under vacuum, were still acceptable at the end of the storage period (13 days).

4. CONCLUSIONS

1. With the species of fish under examination a 100-krad irradiation dose appears sufficient to produce a commercially worthwhile extension of the storage life (10 days from the date of catching). Fish of similar quality to the fresh product is obtained in this way.
2. The use of a 150-krad dose gives an extension of the storage life twice as great as that obtained with 100 krad but the quality of

the product, although acceptable, does not possess freshness criteria adequate for good marketing. The 200-krad dose causes prejudicial changes in the fish, especially in regard to odour, without producing any significant extension of the storage life as compared with the previous dose.

3. In the case of the dose tried (100 krads) packaging under vacuum did not produce any beneficial effect, the biochemical and sensory analysis results being inferior to those obtained in respect of a normal atmosphere. Regarding the packaging material used here (Rilsan), no conclusive results can be quoted since tests on its use are still in the preliminary stages.

ACKNOWLEDGEMENTS

We should like to express our thanks to Mr. Manuel del Val Cob, Head of the Isotopes Section and Mr. Nicasio Ortin Suné, Head of the Isotope Applications Group of the Nuclear Energy Board, for their guidance and support.

Our thanks are also due to our colleagues in the Isotopes Section for their valuable assistance in the performance of the sensory tests.

APPENDIX I

RATING SHEET 1

5-point assessment scale for appearance, odour and texture
of irradiated and control white fish samples
in the raw state

1. Appearance

- 5 — Very brilliant, almost phosphorescent in the light, with no defects (haemorrhage or violet and dark-coloured zones).
- 4 — Brilliant colour but with slight dulling of the lustre as if due to waxing of the surface.
- 3 — Disappearance of characteristic colour of the fish as though discolouration has occurred.
- 2 — Small opaque areas on the surface, presence of defects (haemorrhage, dark or violet-coloured zones).
- 1 — Completely opaque (in the light) and presence of obvious defects.

2. Odour

- 5 — Very good, strong fresh smell as though straight from the sea.
- 4 — Good, no smell.
- 3 — Moderate, slight fishy smell.
- 2 — Limiting quality for consumption, strong smell of fish past its prime.
- 1 — Inedible, strong smell of decomposition or putrefaction (ammonia smell).

3. Texture

- 5 — Firm, elastic reaction to finger pressure.
- 4 — Some loss of elasticity.
- 3 — Loss of firmness or elasticity, but not yet flabby.
- 2 — Flabby or leathery texture.
- 1 — Very flabby, almost spongy or pulpy texture, retains impression of finger pressure.

APPENDIX II

RATING SHEET 2

Sensory evaluation of irradiated raw fish

Name

Date

Parameters \ Samples	Sample No.	Sample No.	Sample No.	Sample No.
Appearance				
Odour				
Texture				

On the basis of your assessment insert the number which you consider appropriate, for each parameter, to each of the samples submitted.

APPENDIX III

RATING SHEET 3

Sensory evaluation of irradiated cooked fish

Name

Date.....

Quality	Sample No. 1				Sample No. 2				Sample No. 3				Sample No. 4			
	Appearance	Texture	Odour	Taste	Appearance	Texture	Odour	Taste	Appearance	Texture	Odour	Taste	Appearance	Texture	Odour	Taste
5. Very good																
4. Good																
3. Medium																
2. Limiting quality for consumption																
1. Unacceptable																
Remarks and suggestions																

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THE BOTULISM HAZARD IN THE PROPOSED USE OF IRRADIATION OF FISH AND FISHERY PRODUCTS IN THE UNITED KINGDOM

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Abstract

THE BOTULISM HAZARD IN THE PROPOSED USE OF IRRADIATION OF FISH AND FISHERY PRODUCTS IN THE UNITED KINGDOM.

Proposals to treat packaged fish with pasteurizing doses of radiation, and the potential health hazards of this process are described. It is proposed to introduce irradiation into an existing processing and distribution chain for retail packs of chilled fish. The Codes of Practice at present in use provide an adequate safeguard against possible health hazards, primarily by the control of temperature, and limit the storage time to about half the potential shelf life of the fish. Irradiation of these products with 0.3 Mrad will result in a considerable extension of storage life whilst maintaining the same high quality of the product. It is concluded that whilst Clostridium botulinum types E, F and non-proteolytic B could be present in the raw material, no particular health hazard exists unless gross malpractice occurs, especially in relation to the temperature of storage.

1. INTRODUCTION

It is now well established that irradiation sterilization of fish and fishery products is meantime not a feasible commercial proposition owing mainly to the undesirable sensory, and in particular flavour, changes produced [1]. On the other hand, irradiation pasteurization, using doses of the order of 200 to 300 000 rads can give considerable extensions, at least two to three times, of the shelf life of a variety of the commoner commercial products, as shown in Table I [2], and yet induce no perceptible alteration in the organoleptic properties.

The problem now is, can this new preservation technique be exploited commercially, either as an alternative or additional process at one or more points in the complex chain of operations from catching fish on board ship until it is sold over the counter to the consumer?

2. POSSIBLE USES OF IRRADIATION

2.1. On board ship

Some attempts have been made in the United States of America [3] and in the Federal Republic of Germany [4] to irradiate newly-caught fish on board ship, and while some of these feasibility studies have not yet been

TABLE I. SOME RELATIONSHIPS BETWEEN ESTIMATED SHELF LIFE OF FISH AND TOXIN PRODUCTION BY Cl. botulinum TYPE E(10⁵–10⁷ spores per gram)

	20°C Shelf life (days)	Toxin (days)	10°C Shelf life (days)	Toxin (days)	5°C Shelf life (days)	Toxin (days)
Cod	1		3	5–9	7	
Cod 0.3 Mrad	2		6–9	5–9	12–15	
Haddock	1		3	5–12	7	
Haddock 0.3 Mrad	2		6–9	5–12	12–15	
Herring	< 1	1–5	1–2	4–7	3–4	12–32
Herring 0.3 Mrad	1	1–5	2–4	4–7	6–8	12–32
Kipper	5–6	2–8	6–9	8–20	7–10	> 28
Kipper 0.3 Mrad	10–15	2–8	10–18	8–20	15–20	> 28
Smoked haddock	2–5	3	5–8	28–30	10–12	> 28
ditto 0.3 Mrad	4–8	3–4	10–15	28–30	20–25	> 28

completed, particularly with regard to X-ray irradiation in the Federal Republic of Germany, we have concluded from the United States work that the use of the more conventional type of irradiator, using say ⁶⁰Co, is not a particularly feasible proposition.

Accordingly, the usefulness of irradiation as a preservation technique appears to be confined to the processing and distribution sections of the industry.

2.2. On shore

A detailed feasibility study on the possible use of the radiation pasteurization of fish and fishery products was carried out in the United Kingdom in 1964 [5] and it was concluded that one of the most likely applications would be in the processing of fresh and smoked fish mainly as fillets, packed in moisture and odour-proof packages to a standard weight, processed at a central depot and distributed through retail outlets such as supermarkets equipped with refrigerated holding stores and display cabinets.

3. PRE-PACKAGING OF FISH

The so-called pre-packaging of fish as envisaged in the above-mentioned feasibility study has been one of the most significant recent developments in fish retailing in the United Kingdom [6–8]. Here a variety of products are either now being sold or are being market-tested. They include fillets of haddock, cod, plaice and sole; smoked products such as kippers and finnan

TABLE II. SHELF-LIFE EXTENSION OF PRE-PACKED IRRADIATED FISH

Number of days on ice before packaging	Shelf life to inedibility (Torry taste-panel score of 4.5)		Shelf life maintaining good quality (Torry taste-panel score of 6.0)	
	Not irradiated (days)	Irradiated (days)	Not irradiated (days)	Irradiated (days)
2	13	30	8	22
4	11	26	6	17
6	9	22	4	11
8	7	18	2	6
10	5	14	0	0
12	3	8	0	0
14	0	0	0	0

haddocks, and breaded fillets of plaice and sole, i.e. fillets which are given a 30-second dip in hot fat at 180°C and then enrobed in bread crumbs. All these products are packed either in polystyrene trays and overwrapped with a plastic film, or placed directly in a plastic pouch, often vacuum-sealed. Such a factory operation would be well suited to the radiation pasteurization of the final packaged products as they came off the packaging line as envisaged in the feasibility study.

An important feature of this marketing system is that it does not utilize the whole shelf life of the packaged fish. In other words, the aim is to market only good-quality fish. In a situation such as exists in the United Kingdom, with a well-developed refrigeration chain, radiation pasteurization could offer considerable advantages to the marketing system while maintaining the good quality of the product over a longer period of time. Packaging itself offers several advantages, such as protection against further contamination, allows for brand identification with all that implies, and for printed instructions to be given with regard to storage and cooking. If radiation pasteurization is superimposed on this system it gives an extension of handling and distribution time while maintaining the same good quality of the product.

There are, however, certain difficulties: packaged fish is just as perishable as unpackaged fish and is certainly likely to be the most perishable food in the supermarket, although the housewife may not realize it. This situation may lead to mishandling in the kitchen and this could be even more likely with irradiated fish.

The kind of advantage envisaged from application of irradiation to packaged fish is illustrated by Table II. In the present distribution system a shelf life of four days is recommended, including the day of packaging. With irradiation the fish would have a shelf life of 11 days to reach the same level

of quality. The proposed system, as can be seen from the table, means that the quality level corresponding to a Torry taste-panel score of 6.0 would represent the worst quality of fish offered for sale. The housewife is at present recommended to consume the fish on the day of purchase or, with refrigeration, on the following day. Such an instruction would still remain on the irradiated fish.

As regards cost, it has been estimated that a plant operating 12 h per day, seven days per week, 50 weeks per year, irradiating fish at 0.3 Mrad with an annual throughput of 2000 tons would add about $\frac{3}{4}$ d./lb to the cost of packaged fish. This estimate includes buildings, plant overheads, depreciation, etc. At 8000 tons per year the additional cost would be $\frac{1}{2}$ d./lb [5].

Experience over the past two or three years in the operation of pre-packaging lines by some of the larger producers in the United Kingdom, using the variety of products already listed above, and operating codes of practice laid down by Torry Research Station in collaboration with the White Fish Authority has shown, through market testing in several supermarket chains that good-quality fish can be successfully marketed in this way.

There is a built-in safety margin so far as the organoleptic properties of the products are concerned, if the codes of practice are operating satisfactorily. It is recognized, however, that difficulties may arise, particularly with regard to temperature control during distribution and marketing, which could be overcome were a greater margin, in terms of shelf life, made available and it is here that it is considered radiation pasteurization could play an important role.

4. Clostridium botulinum POISONING

Apart from the organoleptic considerations, the question of possible food poisoning from botulism has also to be borne in mind in view of the fact that some of the recent serious outbreaks of botulism in the United States of America occurred with packaged smoked fish [9]. Accordingly the following questions have now to be answered:

1. What is the incidence of Cl. botulinum, and in particular of the types capable of growing and producing toxin at chill temperatures, i.e. types E, F, and non-proteolytic B, in the fish landed in United Kingdom ports?

2. If Cl. botulinum were present, what are the hazards for botulinum poisoning in the kinds of operations envisaged by pre-packaging, including distribution and sale in supermarkets?

3. Would irradiation diminish or enhance any botulinum hazard?

5. PRESENCE OF Cl. botulinum IN FISH LANDED IN THE UNITED KINGDOM

Over the past few years the Torry Research Station has made intensive studies in attempts to answer these questions. In a survey of over 600 samples of packaged fish, mainly sold frozen, Cl. botulinum was detected in five

instances [10]. Cl. botulinum type B (non-proteolytic) was encountered in 15 out of 429 samples of bottom deposits from 143 locations round our shores and where fishing operations are continuously taking place [11]. Types E and F were encountered in fish newly caught off our shores on one or two rare occasions. The highest incidence of Cl. botulinum type E occurred in herring landed in the United Kingdom from certain Norwegian fishing grounds [10,12]. Estimations of the numbers present, using the Most-Probable Number technique, showed about one spore per 16 g of fish or less [10]. This compares with a value of 17 per 100 g of fish in commercially produced haddock fillets in the United States of America [13].

It must be assumed, therefore, that Cl. botulinum types capable of growing at chill temperature could be present on our fish, albeit at low levels of infection, either as a natural infection or from contamination during processing.

6. GROWTH OF Cl. botulinum IN PRE-PACKAGED FISH

Assuming infection by Cl. botulinum, the question now is what are the hazards from botulism with pre-packaged fish?

In the codes of practice already mentioned it is stipulated that the temperature of the fish should never exceed 3°C during processing, packaging, distribution, storage and display. This is because it is known that the minimum temperature for the growth of Cl. botulinum, types E, F and non-proteolytic B, is about 3.8°C [14]. No botulism hazard exists therefore if these codes of practice are adhered to.

In the case of smoked fish and breaded fish products the temperature of 3°C will inevitably be exceeded during processing.

Cold smoked products such as kippers and finnans reach temperatures of about 15°C for 3–4 hours in a modern smoking kiln. In addition, however, they are given a preliminary brining process resulting in a salt content in the flesh of 2–3%, and the fish is impregnated with anti-bacterial components from the smoke itself. Whilst neither of these factors alone, or in combination, is sufficient to kill Cl. botulinum spores, the data available show some inhibition of growth and toxin production, at least in the case of type E.

Hot smoked products reach temperatures of about 60°C for 1/2–2 hours; this could inactivate some toxin but would not necessarily kill all the spores present. The concentration of salt and smoke constituents is somewhat higher in this type of product than in cold smoked fish.

In the case of breaded fish products a 30-second exposure to 180°C would raise a significant portion of the fish about 3°C, although the exact time and temperature conditions have not been determined. With efficient processing and subsequent cooling, however, the time of exposure to the higher temperatures should be relatively short.

It seems certain therefore that neither the smoking nor breading process provide suitable conditions for the development of significant amounts of toxin. On the other hand it is certain that spores would not necessarily be killed.

It has already been stated that Cl. botulinum types E, F and non-proteolytic B do not grow or produce toxin at temperatures below 3.8°C. Even at higher chill temperatures up to, say, 5° or 6°C, although growth could occur, it is relatively slow and all our data seem to show that the pre-packaged products already mentioned would be spoiled and become organoleptically unacceptable before growth and toxin formation could occur (Table I). At higher temperatures, especially about ambient temperature, toxin formation could occur before the end of shelf life was reached (Table I) [15].

The proposed marketing system, as already stated, does not utilize the whole of the potential shelf life of fishery products; this, along with the proposed codes of practice, should ensure an adequate safety margin both from the point of view of spoilage and risk of botulism. It is therefore important to continuously impress upon the housewife that pre-packaged fish must not be allowed to lie around a warm kitchen but should be consumed on the day of purchase or kept refrigerated for no longer than one further day.

Almost all pre-packaged fish products would be cooked before consumption and this in itself is sufficient to destroy any preformed Cl. botulinum toxin since the data available show that type E toxin is destroyed after 5-minutes heating at 65°C [16, 17]. A popular pre-packed fish product in the United Kingdom is the so-called "boil-in-the-bag" smoked fish (kippers or finnans) which are at present sold usually in the frozen state. Such packs were experimentally inoculated with 10^6 spores per 100 to 200 g of fish (i.e. much in excess of that encountered as a natural infection) and stored for 6 days at 30°C. They were then boiled in the manner specified by immersing in an average-sized domestic pan of boiling water, brought to the boil and the botulinum toxin assayed at various periods. The results showed that no toxin remained after 5 minutes boiling [17]. It is concluded, therefore, that the hazard from botulinum poisoning from pre-packaged fish which is normally cooked before consumption is negligible.

Kipper fillets may be eaten raw as a savoury on toast and smoked salmon is normally eaten uncooked and here there are potential hazards from botulinum poisoning if the products are mishandled at any point from production to consumption.

7. GROWTH AND TOXIN PRODUCTION OF Cl. botulinum IN IRRADIATED FISH

The remaining question now to be answered is would irradiation of pre-packaged fishery products enhance or diminish the botulism hazard? All the data now available, both from the United Kingdom and the United States of America, seem to indicate that with many species of fish after irradiation and during subsequent storage there is an enhanced risk of botulism [15, 18]. Thus, we have found that with cod and plaice, despite some variations, toxin production was quicker after irradiation at 0.3 Mrad and that with herring toxin production, once it began, was quicker and reached a higher titre in the irra-

diated fish [18]. Somewhat similar results have been obtained the United States of America with haddock and sole [15]. The most disturbing feature, particularly noticeable in the United States results, is that the product frequently became toxic when stored at 5.5 to 7.2°C before it was considered no longer edible by normal sensory assessment.

Now several laboratories have observed that growth and toxin production with Cl. botulinum (type E) varies considerably with species of fish used. Thus, herrings are known to be very botulinogenic and this was attributed to their high lipid content. It has more recently been suggested, however, that it is due to the high glucose and ribose content of the flesh, because by diminishing the amounts of these sugars by the use of an enzyme preparation, the toxin content is lowered by about 40 times that of the control [15]. Radiation pasteurization of fish has, therefore, a two-fold effect:

1. It kills off the spoilage bacteria and so extends the shelf life.
2. By reducing the bacterial flora which normally would utilize any carbohydrate such as glucose or ribose in the flesh, it can result in an increased toxin titre, if the organism is present and the time temperature conditions are suitable. This also explains why at irradiation doses of say 0.1 Mrad, where the bacterial kill-off is not so great as with 0.3 Mrad, there does not appear to be any enhanced production of botulinum toxin.

8. CONCLUSIONS

Cl. botulinum types E, F, or non-proteolytic B may be present in fish landed in the United Kingdom, although the level of infection is never likely to exceed 12 or 15 spores per 100 g of flesh.

If good commercial practice is followed in the processing line, during distribution until consumption, and in particular if the temperature of the produce is never allowed to exceed 3.0°C, there should be no danger from botulinum poisoning, even if irradiation were employed. Even if some toxin were to be formed by malpractice at any stage during the distribution chain, including the consumer's kitchen, subsequent cooking would almost certainly destroy any toxin present. Only when products such as smoked herring fillets or smoked salmon are eaten raw would the potential hazard become real.

This paper was prepared as part of the program of the Torrey Research Station.

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THE SIGNIFICANCE OF NON-PROTEOLYTIC Clostridium botulinum TYPES B, E AND F IN THE DEVELOPMENT OF RADIATION- PASTEURIZED FISHERY PRODUCTS

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Abstract

THE SIGNIFICANCE OF NON-PROTEOLYTIC Clostridium botulinum TYPES B, E AND F IN THE DEVELOPMENT OF RADIATION-PASTEURIZED FISHERY PRODUCTS.

The outgrowth and toxin production of Clostridium botulinum type E has been studied in unirradiated and irradiated Atlantic Coast haddock and cod and Pacific Coast English, petrale, and Dover sole fillets stored in the temperature range of 38 to 50°F (3.3 to 10°C). Data collected thus far suggest that irradiated haddock would not provide any more of a health hazard than unirradiated haddock provided the product could be distributed and stored at 42°F (5.6°C) or below. At 46°F (7.8°C) storage, the inoculated pack results with 100-krad irradiation appear favourable, since there is a considerable safety margin between maximum storage life and earliest toxin production by type E. The samples irradiated at 200 krad appear less favourable because of the longer storage life and earlier toxin development of toxin in samples inoculated with 10⁴ spores/g. At 50°F (10°C) storage, a safety margin does not exist between maximum storage and toxin production for either the unirradiated or irradiated samples. More detailed experiments are currently in progress to evaluate the degree of safety that exists for irradiated haddock stored at different temperatures. Irradiated petrale, English, and Dover sole (from the Pacific Coast of the United States) are better substrates for Cl. botulinum than either haddock fillet. Outgrowth and toxin production occurred in the irradiated sole species vacuum-packaged, but not in samples packaged in oxygen-permeable films stored at 42°F (5.6°C). It is conjectured that the ribose and glucose contents of the fish fillets are important factors which account for the differences in the rates of toxin production in different species of fish and in English sole fillets packaged in oxygen-permeable or impermeable films. Non-proteolytic Cl. botulinum types B and F, isolated at our laboratory from marine sediments, share the same physiological and biochemical characteristics possessed by type E. These cultures all grow at refrigerated temperatures as low as 38°F (3.3°C) and produce toxin. Experiments are planned to compare the outgrowth time and toxin production of the type B and F spores in unirradiated and irradiated haddock stored at 38 to 50°F (3.3 to 10°C). The relationship of bacteriophages (bacterial viruses) of Cl. botulinum to the toxigenicity of the bacterium is currently being studied. Bacteriophages have been observed in electron micrographs of the lysates of the different types of Cl. botulinum. Bacteriophages of non-proteolytic types B and F and proteolytic type F have been propagated and a study of the relationship of these phages to the toxigenesis of Cl. botulinum is currently in progress.

1. INTRODUCTION

Low doses of ionizing radiation have been shown to be effective in inactivating large percentages of bacteria on fish fillets and other foods of marine origin. Consequently, the storage life of the irradiated product is extended at refrigerated temperatures. The irradiation doses employed for pasteurization not only reduce the total bacterial population, but selectively eliminate the Pseudomonas species (the predominant bacteria on spoiled unirradiated fish fillets).

The inactivation of the pseudomonads not only delays the onset of spoilage but in many cases changes the type of spoilage common to unirradiated fish.

The extension in the storage life of irradiated fish and the changes in the spoilage patterns of irradiated fish could possibly create a public health problem due to Clostridium botulinum. This reasoning is based on:

- (i) The ability of non-proteolytic types B, E and F to grow and produce toxin at refrigerated temperatures (Eklund et al. [1, 2] and Schmidt et al. [3]).
- (ii) Irradiation doses considered for pasteurizing fishery products (doses under 500 krad) have no major destructive effects on spores of this organism (Schmidt et al. [4]).
- (iii) Recent ecological surveys in the United States and elsewhere have shown the occurrence of these types in the marine environment.

The work reported here has been directed to the question of Cl. botulinum and its significance as a possible public health hazard in radiation-pasteurized fish. It covers three areas of research as follows:

- I. Toxin production by Cl. botulinum type E in unirradiated and irradiated fish fillets.
- II. Characteristics of non-proteolytic Cl. botulinum types B and F and their possible importance in fishery products preserved by ionizing radiation.
- III. Bacteriophages of the different types of Cl. botulinum.

2. TOXIN PRODUCTION BY Cl. botulinum TYPE E IN UNIRRADIATED AND IRRADIATED FISH FILLETS

The maximum storage life data presented in this report was collected by the Bureau of Commercial Fisheries (BCF) Technology Laboratories in Seattle, Washington; Pascagoula, Mississippi; and Gloucester, Massachusetts; and by the Food Science Department, Michigan State University, East Lansing, Michigan.

Toxin production by Cl. botulinum type E was determined in unirradiated and irradiated (1) haddock (Melanogrammus aeglefinus), (2) English sole (Parophrys vetulus), (3) Dover sole (Microstomus pacificus), (4) petrale sole (Eopsetta jordani), and (5) cod (Gadus morhua) fillets.

2.1. Procedure

The extent of toxin production was determined in sealed packages of fresh fish fillets which were inoculated with type-E spore suspensions, irradiated, and stored under various conditions prior to assay.

2.1.1. Inoculated packs

Freshly caught haddock or cod were filleted, packed in ice, and shipped by air freight to the different laboratories from Gloucester, Massachusetts. Petrale, English, and Dover sole were purchased from local processors located on the Pacific Coast of the United States. At the laboratory, the fillets were cut into samples with a total weight of about 50 g. The fillets were then packaged with oxygen-permeable films (polyethylene film) or oxygen-impermeable films (mylar-polyethylene). The mylar-polyethylene bags contained a rubber septum held to it with an adhesive. This enabled us to vacuum-pack the fillets with commercial packaging machines. The packages were then taken into the botulinum laboratory and the inoculum of a type-E spore suspension was injected by syringe and needle through the rubber septum into multiple sites of the fillet. Fillets packaged in polyethylene bags were inoculated through the bag opening and then each package was heat-sealed.

After inoculation, all the samples were stored in ice until irradiated.

2.1.2. Preparation of type-E spore suspensions

Spores of the Beluga strain of type E were produced in TPG (trypticase, peptone, glucose) medium at 30°C as described by Eklund and Poysky [5]. Viable spore counts were determined at 25°C using TPG and peptone yeast-extract agar. Sodium thioglycollate was added as the reducing agent in the different media. The spore suspensions were diluted so that 0.5 ml of inoculum would contain the desired concentration of spores.

2.1.3. Irradiation

The samples were irradiated with cobalt-60 sources. After irradiation, the samples were stored at different temperatures ranging from 38 to 50°F.

2.1.4. Toxin assays

Triplicate samples were removed from storage at intervals of 3 to 4 days and assayed for botulinum toxin. Each sample (50 g) was lightly blended with 100 ml of pre-chilled physiological saline. The homogenate was then

transferred to centrifuge tubes and centrifuged at 5000 rev/min. The supernatant was removed and 0.4 ml injected intra-peritoneally into a pair of Swiss Webster mice. The samples were assayed for toxin in both the trypsinized and untrypsinized states. The trypsin-digestion procedure includes the adjustment of the supernatant to pH 6.0; the addition of Difco 1:250 (10 per cent) trypsin at a final concentration of 0.5 per cent; and incubation at 37°C for 1 hour.

Samples that were toxic to mice within 72 hours were confirmed as containing type-E toxin by the mouse-protection test. For the mouse-protection test, 0.4 ml of sample supernatant was mixed in vitro with 0.1 ml of type-E anti-toxin and allowed to react at 25°C for 1 hour. Each pair of Swiss Webster mice was then injected with 0.5 ml of the mixture. If the type-E-protected mice lived and the unprotected mice died with characteristic symptoms of botulism, the samples were confirmed as containing type-E toxin. In many instances, the mouse-protection test was run at the same time that the unprotected mice were injected with the sample supernatant.

Occasionally mouse deaths occurred by factors other than botulinum toxin. A considerable amount of this toxicity could be prevented by injecting antibiotics into the mice as recommended by Segner et al. [6]. Each antibiotic injection consisted of 0.1 ml volume containing 5 mg of chloromycetin and 0.625 mg of terramycin.

2.2. Results and discussion

The concept of the inoculated-pack studies is to determine whether irradiation preservation of fishery products could create more favourable conditions for the growth and toxin production of *Cl. botulinum* than exists in unirradiated fish fillets. This involves the determination of the maximum expected storage life and the earliest time of type-E toxin production in haddock, cod, Dover, English, and petrale sole fillets stored in the temperature range of 38°F (3.3°C) to 50°F (10°C). The acceptance or rejection of a sample is based mainly on odour of the raw fish¹. A more detailed description of the procedures used to establish the maximum storage life will be discussed by Mr. Ronsivalli at this meeting².

2.3. Comparison of maximum storage life and earliest toxin production by type E in unirradiated and irradiated haddock fillets

The outgrowth and toxin production by type-E spores in unirradiated and irradiated haddock fillets have been studied at storage temperatures of 50, 46, and 42°F (10, 7.8, and 5.6°C).

1 The term maximum storage life used throughout this report refers to the point during storage at which all members of a consumer-type panel rejected the fillets as spoiled, based primarily on odour.

2 See: RONSIVALLI, L.J. et al., "Studies in petition-oriented aspects of radiation pasteurization of fishery products", these Proceedings, PL-319/1.

2.3.1. Storage at 50°F (10°C) in oxygen-permeable packages

The results of these studies are summarized in Fig. 1. With an inoculum of 10^2 spores/g, toxin was detected in both unirradiated and irradiated samples after 11 days of storage. When the inoculum level was increased to 10^4 spores/g, toxin was detected earlier in samples irradiated at 200 krad than in 100 krad or the unirradiated samples. Toxin was present in the unirradiated and irradiated samples before the maximum storage life of the fillets. The maximum storage life was longest in samples irradiated at 200 krad.

2.3.2. Storage at 46°F (7.8°C) in oxygen-permeable packages

The results of these inoculated pack studies are summarized in Fig. 2. Toxin was detected in fillets inoculated with 10^2 spores/g after the maximum storage life of the unirradiated and 200-krad samples. Toxin was not detected

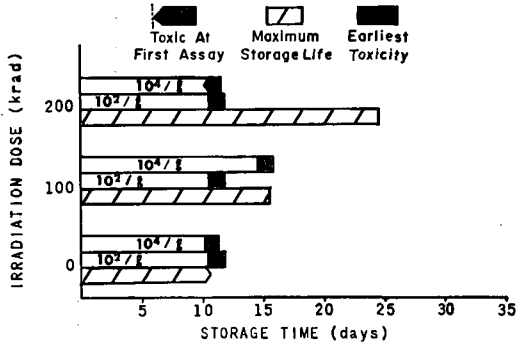


FIG. 1. Comparison of maximum storage life and toxin production by *Cl. botulinum* (10² and 10⁴ spores per gram) in irradiated and unirradiated haddock fillets (packaged in oxygen-permeable films) stored at 50°F (10°C).

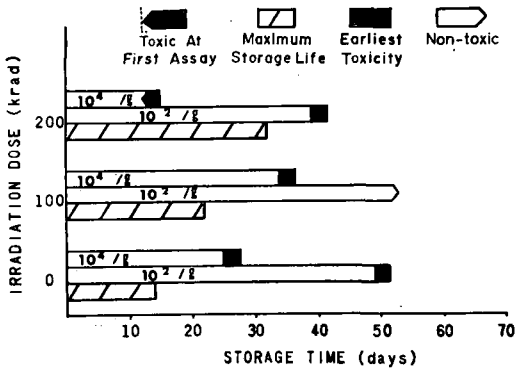


FIG. 2. Comparison of maximum storage life and toxin production by *Cl. botulinum* type E (10² and 10⁴ spores per gram) in irradiated and unirradiated haddock fillets (packaged in oxygen-permeable films) stored at 46°F (7.8°C).

in the 100-krad samples during the 55-day storage period. With an inoculum level of 10^4 spores/g, toxin was detected earlier in the 200-krad samples than in either the 100-krad or unirradiated samples. Toxin was detected at a later storage period in samples irradiated at 100 krad than in the unirradiated samples. The margin of safety between toxin production and maximum storage life is therefore greatest in samples irradiated at 100 krad.

2.3.3. Storage at 42°F (5.6°C) in oxygen-permeable packages

The results from studies conducted at 42°F (5.6°C) were somewhat different from those obtained at the higher storage temperatures in that toxin was not detected in any of the unirradiated or irradiated samples inoculated with 10^4 spores/g (see Fig. 3). These data indicate that a considerable degree of safety exists for irradiated haddock fillets stored at 42°F (5.6°C). Toxin was detected in the unirradiated and irradiated haddock fillets when the inoculum was increased to 10^6 spores/g. The toxin titres, however, did not increase during storage, therefore it is entirely possible that with such a massive inoculum germination occurred and toxin was released without subsequent growth.

2.3.4. Storage at 42°F (5.6°C) in oxygen-impermeable packages

Figure 4 summarizes the results from the inoculated-pack studies with haddock packaged in oxygen-impermeable packages. The only experiments completed are those in which the fillets were inoculated with 10^4 spores/g. None of the samples contained detectable levels of botulinum toxin during the 55-day storage period. Although the experiments are relatively few, we do not find any differences between the outgrowth and toxin production of type-E spores in haddock packaged in oxygen-permeable or impermeable films. The only obvious difference is the longer maximum storage life of samples packaged in oxygen-impermeable films.

2.3.5. Evaluation of data on haddock fillets

Data collected thus far suggest that irradiated haddock would not provide any more of a health hazard than unirradiated haddock provided the product could be distributed and stored at 42°F (5.6°C) or below.

At 46°F (7.8°C) storage, the inoculated-pack results with 100-krad irradiation appear favourable, since there is a considerable safety margin between maximum storage life and toxin production by type E. The samples irradiated at 200 krad appear less favourable because of the longer maximum storage life and earlier development of toxin in samples inoculated with 10^4 spores/g. At 50°F (10°C) storage, a safety margin does not exist for either the unirradiated or irradiated samples when inocula of 10^2 or 10^4 spores/g are used.

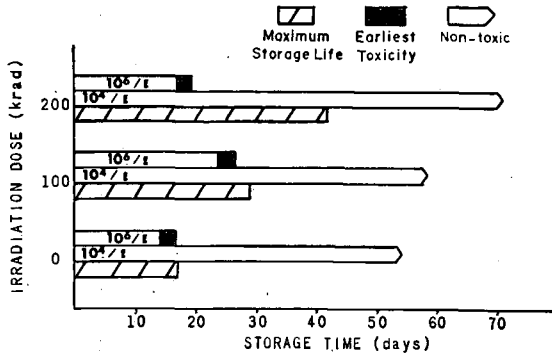


FIG. 3. Comparison of maximum storage life and toxin production by *Cl. botulinum* type E (10^4 and 10^6 spores per gram) in irradiated and unirradiated haddock fillets (packaged in oxygen-permeable films) and stored at 42°F (5.6°C).

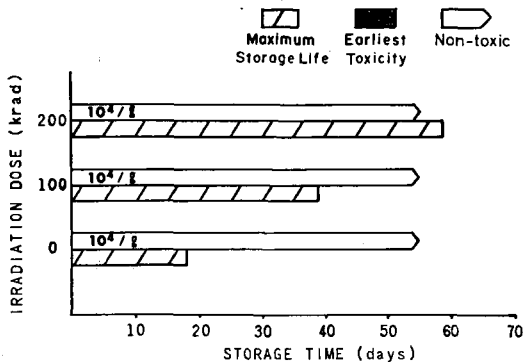


FIG. 4. Comparison of maximum storage life and toxin production by *Cl. botulinum* type E (10^4 spores per gram) in irradiated and unirradiated haddock fillets (packaged in oxygen-impermeable films) and stored at 42°F (5.6°C).

2.4. Comparison of the maximum storage life and earliest toxin production by type E in unirradiated and irradiated Dover sole fillets

Dover sole fillets were packaged in oxygen-impermeable films and were stored at 42 and 46°F (5.6 and 7.8°C).

Previous studies in our laboratory have shown that rancidity develops rapidly in irradiated Dover, English, and petrale sole fillets packaged in oxygen-permeable films. Therefore, these fillets were vacuum-packed prior to irradiation.

2.4.1. Storage at 46°F (7.8°C)

Inoculum levels of 10^2 and 10^4 spores/g were used in these experiments (see Fig. 5). Toxin was not detected in the unirradiated samples inoculated with 10^2 spores/g; however, after 36 days of storage toxin was present in samples inoculated with 10^4 spores/g. Irradiated samples became toxic with either the 10^2 or 10^4 /g spore inoculum. Toxin developed after the maximum storage life in the 100-krad samples but before the maximum storage life in the 200-krad samples.

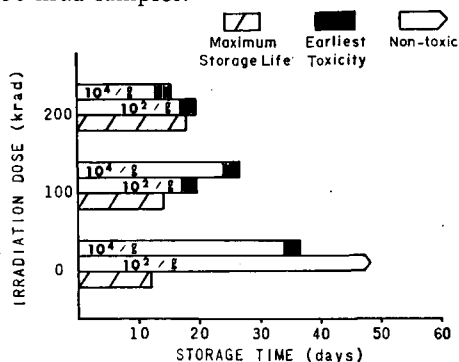


FIG. 5. Comparison of maximum storage life and toxin production by *Cl. botulinum* type E (10^2 and 10^4 spores per gram) in irradiated and unirradiated Dover sole fillets (packaged in oxygen-impermeable films) and stored at 46°F (7.8°C).

2.4.2. Storage at 42°F (5.6°C)

When the inoculated samples were stored at 42°F (5.6°C), none of the unirradiated samples contained type-E toxin during the 53-day storage period (see Fig. 6). Toxin was, however, detected in the 100- and 200-krad samples inoculated with either 10^2 or 10^4 spores/g. The differences between maximum storage life and earliest toxin production decreased as the irradiation dose increased.

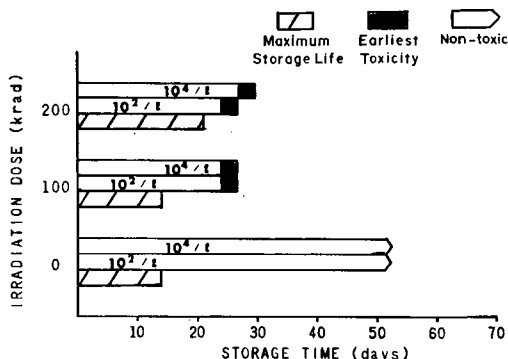


FIG. 6. Comparison of maximum storage life and toxin production by *Cl. botulinum* type E (10^2 and 10^4 spores per gram) in irradiated and unirradiated Dover sole fillets (packaged in oxygen-impermeable films) and stored at 42°F (5.6°C).

2.5. Comparison of the maximum storage life and earliest toxin production by type E in unirradiated and irradiated English sole fillets

The main purpose of these experiments was to determine whether irradiated fillets of other fish species would be comparable to Dover sole in supporting the outgrowth and toxin production of type-E spores. The results of these studies are presented in Fig. 7.

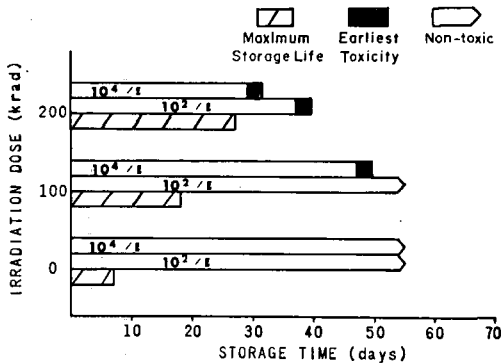


FIG. 7. Comparison of maximum storage life and toxin production by *Cl. botulinum* type E (10^2 and 10^4 spores per gram) in irradiated and unirradiated English sole fillets (packaged in oxygen-impermeable films) and stored at 42°F (5.6°C).

2.5.1. Storage at 42°F (5.6°C)

The results obtained for English and Dover sole fillets were very similar in that toxin was not detected in the unirradiated samples.

Toxin production occurred earlier in the samples irradiated at 200 krad than in the 100-krad samples. The margin of safety between maximum storage life and toxin production decreased with increasing doses of irradiation. This was the result of a longer maximum storage life and earlier toxin production of the irradiated samples.

2.6. Comparison of the maximum storage life and earliest toxin production by type E in unirradiated and irradiated petrale sole fillets

Petrable sole fillets were vacuum-packaged and stored at 38 and 42°F. (3.3 and 5.6°C) in the initial experiments. An additional experiment was conducted at 42°F (5.6°C) to evaluate package and inocula variables.

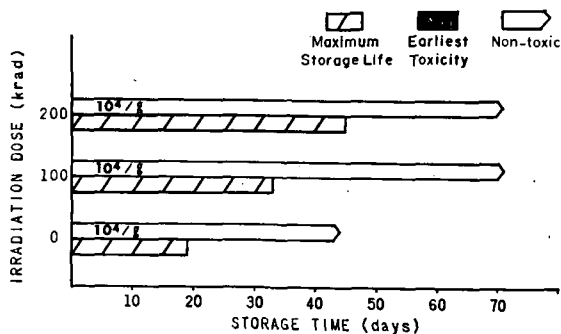


FIG. 8. Comparison of maximum storage life and toxin production by *Cl. botulinum* type E (10^4 spores per gram) in irradiated and unirradiated petrale sole fillets (packaged in oxygen-impermeable films) and stored at 38°F (3.3°C).

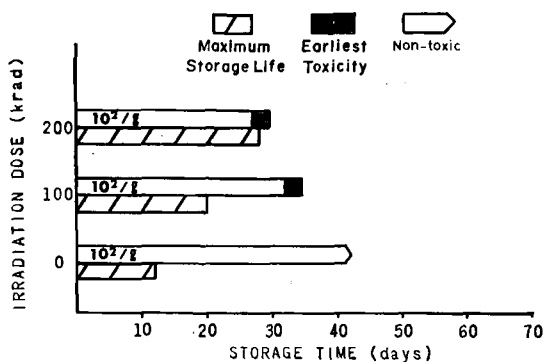


FIG. 9. Comparison of maximum storage life and toxin production by *Cl. botulinum* type E (10^2 spores per gram) in irradiated and unirradiated petrale sole fillets (packaged in oxygen-impermeable films) and stored at 42°F (5.6°C).

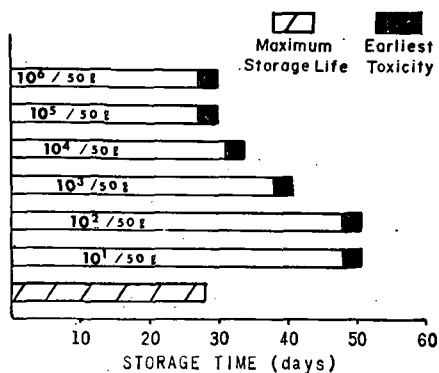


FIG. 10. Toxin production by different concentrations of type E (spores per 50 gram) inoculated into petrale sole fillets, irradiated at 200 krad, and stored at 42°F (5.6°C); packaged in oxygen-impermeable films.

2.6.1. Storage at 38°F (3.3°C)

Figure 8 summarizes the results of these experiments in which a single level of inoculum at 10^4 spores/g was used. None of the irradiated or un-irradiated samples contained type-E toxin at 38°F (3.3 °C).

2.6.2. Storage at 42°F (5.6°C)

Figure 9 shows that irradiated petrale sole fillets with an inoculum of 10^2 spores/g were comparable to English and Dover sole in supporting the outgrowth and toxin production by type-E spores at 42°F (5.6°C).

Effect of spore concentration. Therefore, an experiment was designed to determine the rate of outgrowth and toxin production in petrale sole fillets inoculated with different concentrations of type-E spores. All of the samples were vacuum-packaged and irradiated at 200 krad. Toxin production by the different spore concentrations are presented in Fig. 10. The storage time for toxin production increased as the spore inoculum decreased. Toxin was produced even when the inoculum was 10 spores/50 g fillet.

Effect of vacuum-packaging. A comparison was also made of the rate of outgrowth and toxin production in irradiated petrale sole fillets packaged in oxygen-permeable and -impermeable films. The comparative results of this experiment are shown in Fig. 11. The fillets packaged in oxygen-permeable films were non-toxic after 43 days of storage at 42°F (5.6°C). In contrast, the vacuum-packaged samples were toxic after 32 days of storage.

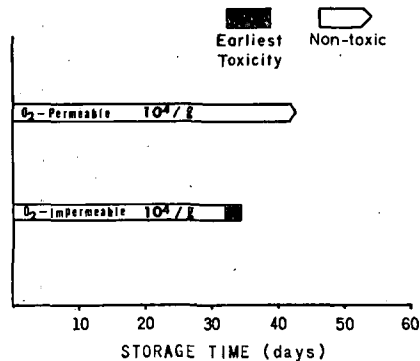


FIG. 11. Toxin production of type E (10^4 spores per gram) in irradiated (200 krad) petrale sole fillets packaged in oxygen-impermeable and oxygen-permeable films and stored at 42°F (5.6°C).

2.7. Comparison of toxin production by type E in different fish fillets

The rate of toxin production of type-E spores in unirradiated and irradiated haddock and petrale, English, and Dover sole fillets is shown in Table I.

TABLE I. TOXIN PRODUCTION BY *Clostridium botulinum* TYPE E (10^4 spores/g) IN DIFFERENT VACUUM-PACKED FISH SPECIES, IRRADIATED AT 100 AND 200 kradS AND STORED AT 42°F (5.6°C)

Fish	Irradiation dose (krads)		
	0	100	200
	Earliest detection of toxin (days)		
Haddock	> 55	> 55	> 55
Petrale sole	> 35	35	23
Dover sole	> 42	24	27
English sole	> 55	47	29

It is evident that the irradiated petrale, Dover, and English sole fillets support the rapid outgrowth and toxin production of type-E spores. Toxin, however, was not detected in any of the unirradiated or irradiated haddock fillets during the 55 days in storage at 42°F (5.6°C). Toxin was detected earlier in the English and petrale sole fillets irradiated at 200 kradS than in the 100-krad or unirradiated samples.

The inoculated-pack protocol followed in these experiments definitely has certain drawbacks. When the spores are inoculated into the fish fillet, much of the inocula weeps out of the injection site and accumulates in the drip within the package. Thus, there is a very uneven distribution of spores.

Secondly, the massive numbers of spores used could be regarded as very unrealistic. We have detected toxin in samples inoculated with 10^6 heat-shocked spores (140°F, 30 minutes) very early in storage without subsequent increases in toxin titres. The question therefore arises as to whether germination alone may have occurred without subsequent growth. Spores will germinate in TPG medium at 32°F (0°C) without subsequent growth. It is difficult to assess the safety of the irradiated products, such as haddock, because the unirradiated samples inoculated with such high spore concentrations in many cases contain toxin after short periods of storage. We hope that a number of these factors can be discussed at this meeting because the protocol for assessing the public-health safety of a new food process or product relative to toxin production by *Cl. botulinum* needs to be markedly improved.

2.8. A conjecture of some of the factors responsible for the differences of rate of toxin production in unirradiated and irradiated fish fillets

In earlier parts of this report, we have stated that (i) different species of fish, (ii) permeability of packages, (iii) irradiation, and (iv) storage temperatures influence the rate of toxin production by *Cl. botulinum* type E. The

reasons for the apparent increased rate of toxin production in irradiated fish and the different species of sole packaged in oxygen-impermeable films is unknown. The production of proteolytic enzymes by the microflora undoubtedly plays an important role in the destruction of the toxin in unirradiated fish fillets. An equally important factor is the competitive microflora which, among other activities, depletes the available nutrients, especially carbohydrates.

The predominant spoilage floras on irradiated petrale sole fillets packaged in oxygen-permeable films are yeasts and Achromobacter. None of these oxygen-permeable packaged fillets have contained detectable levels of botulinum toxin at 42°F (5.6°C).

When the fillets are vacuum-packaged, the predominant spoilage flora is lactobacilli. These vacuum-packaged fillets also support the outgrowth and toxin production of type-E spores. During the course of these studies, Mr. John Spinelli from our laboratory observed a considerable difference in the carbohydrate content of irradiated and unirradiated English sole fillets.

Figures 12 and 13 are presented at this point to compare the ribose and glucose contents in unirradiated and irradiated English sole fillets packaged in oxygen-permeable films.

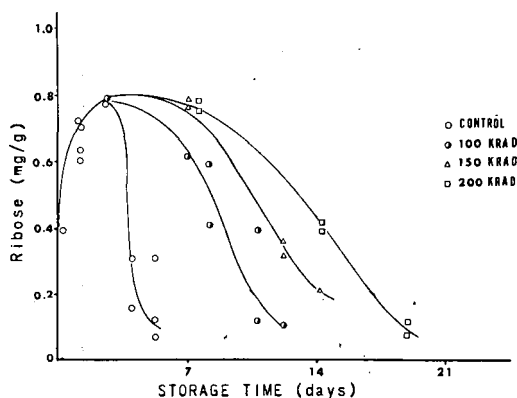


FIG. 12. Ribose in English sole fillets irradiated at 0, 0.1, 0.15, and 0.2 megarad and stored at 42°F (5.6°C); air-packed.

In unirradiated English sole fillets both the glucose and free ribose decrease very rapidly from their maximum concentrations. Irradiation causes a decrease in the rate of disappearance. The changes in ribose and glucose content in unirradiated and irradiated vacuum-packaged English sole fillets are shown in Figs 14 and 15.

When vacuum-packaged, the ribose content of the irradiated fillets decreased very slowly. In fact, the ribose content of the 200-krad samples remained fairly constant during the first 20 days of storage. The glucose content

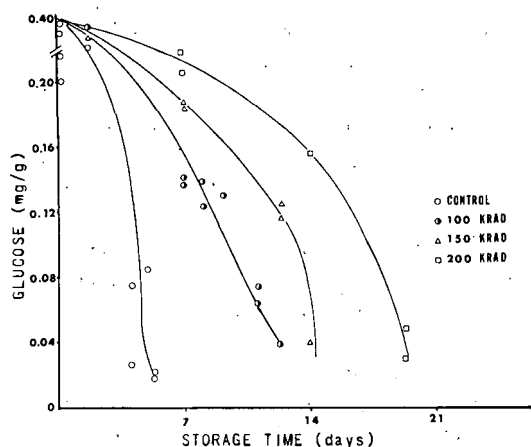


FIG. 13. Glucose in English sole fillets irradiated at 0, 0.1, 0.15, and 0.2 megarad and stored at 42°F (5.6°C); air-packed.

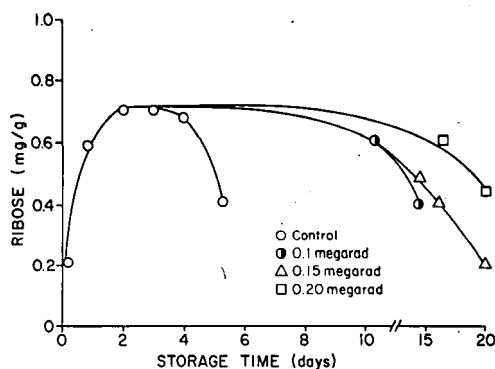


FIG. 14. Free ribose in vacuum-packed English sole fillets irradiated at 0, 0.1, 0.15, and 0.2 megarad and stored at 42°F (5.6°C).

of vacuum-packaged fish fillets was initially lower than the glucose content of the air-packed samples because a different lot of fish was used. Even within a given lot of fish, the glucose content varies markedly. The glucose in English sole decreased rapidly in both air-packed and vacuum-packed samples, but the rate was slower with the irradiated samples.

An experiment was therefore started to determine the outgrowth and toxin production of type-E spores in trypticase-peptone medium with and without glucose and ribose. Table II summarizes the results of these experiments. Without a carbohydrate source, both growth and toxin were absent in the tubes incubated at 42°F (5.6°C). Low levels of toxin were detected in the tubes incubated at 50°F (10°C) but there was no evidence of growth.

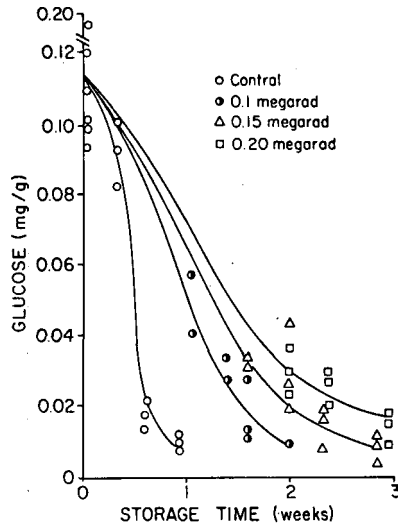


FIG. 15. Free glucose in vacuum-packed English sole fillets irradiated at 0, 0.1, 0.15, and 0.2 megarad and stored at 42°F (5.6°C).

TABLE II. EFFECT OF CARBOHYDRATE AND CARBOHYDRATE CONCENTRATIONS ON TOXIN PRODUCTION BY *Cl. botulinum* TYPE E (10^4 spores/tube) IN TRYPTICASE-PEPTONE MEDIUM

Carbohydrate concentration	Toxin titre after incubation at: ^a	
	42°F (5.6°C)	50°F (10°C)
	(MLD/ml)	(MLD/ml)
TP base only	< 20	20
Glucose 0.1%	100	2 000
Glucose 0.2%	200	20 000
Ribose 0.1%	200	10 000
Glucose 0.1%, ribose 0.1%	1 000	20 000

^a I.P. assay - trypsinized state after 54 days at 42°F (5.6°C) and 25 days at 50°F (10°C).

When glucose, ribose, and a mixture of the two carbohydrates were present, good growth was observed and appreciable amounts of toxin were present. Higher toxin titres were obtained at 50°F (10°C) than at 42°F (5.6°C).

The toxin titres were generally higher in tubes containing ribose than those containing glucose. It is also interesting to note that the toxin titres were higher at 42°F (5.6°C) when both glucose and ribose were present.

TABLE III. EFFECT OF GLUCOSE AND RIBOSE ON TOXIN PRODUCTION BY *Cl. botulinum* TYPE E (10^4 spores/g) IN VACUUM-PACKAGED HADDOCK AND COD FILLETS IRRADIATED AT 200 kradS AND STORED AT 42°F (5.6°C)

Treatment	Earliest toxin production in:	
	Haddock	Cod
	(Days)	(Days)
No carbohydrate added	> 40	> 40
Ribose 10 μ M/g	> 40	20
Glucose 10 μ M/g	> 40	26

The next experiment of this series was done to determine the effect of carbohydrates on toxin production by type-E spores in haddock and cod fillets. Ribose or glucose was injected into the fillets which were stored in oxygen-impermeable films overnight on ice. The following morning the packages were inoculated with 10^4 spores/g and all samples were irradiated at 200 kradS and stored at 42°F. Toxin was not detected in any of the haddock fillets during the 40-day storage period. Cod fillets without added carbohydrate also were non-toxic after 40 days of storage, but the fillets with added ribose were toxic in 20 days, and with added glucose, they were toxic in 26 days (see Table III).

Weimberg [7] reported that a particulate enzyme from *Pseudomonas fragi* (a micro-organism commonly found in fish) oxidizes ribose to its gamma-lactone. Leroux and Tarr [8] using the same organism demonstrated that the particulate fraction possessed both powerful ribose and glucose dehydrogenases which oxidized D-ribose to D-ribono-gamma-lactone and glucose to glucono-delta-lactone. The enzyme system was used by von Tigerstrom and Tarr [9] to prevent browning in fish muscle to be dried or canned. When crude preparations of these enzymes were incorporated into intact and minced fish tissue, the carbohydrate levels could be significantly lowered by the treatment. Reduction of the carbohydrate level in intact fish tissue took somewhat longer than in the minced product. It seems probable therefore that fish fillets can be treated with enzyme systems to lower their carbohydrate content and consequently prolong the outgrowth and toxin production time of type E.

Enzyme systems have been successfully used to reduce the carbohydrate levels in different foods (e.g. glucose is normally removed from egg white prior to drying by use of glucose oxidase).

An experiment was done to determine whether enzyme systems of *Ps. fragi* could be used to lower the ribose and glucose content in solution and what effect this solution with added nutrient had on the toxin production of type E at 60°F (15.6°C).

2.8.1. Procedures

The particulate enzyme used in this experiment was prepared by the method of Weimberg [7].

Duplicate portions of the following mixture were each incubated at 25°C (77°F): 30 ml of 0.004M MgSO₄; 30 ml of 0.01M potassium phosphate buffer, pH 7.0; 4.5 ml of particulate enzyme preparation; and glucose or ribose to a final concentration of 0.1 per cent. After mixing, 30 ml of the mixture was immediately removed and heated in a boiling water bath to inactivate the enzymes (this served as the untreated control). The remainder of the mixture was incubated at 25°C (77°F) and an aliquot of the solution was removed every 2 hours for analysis of free sugars. To determine free sugars, the solutions were de-proteinized with 10 per cent TCA and clarified by filtration. Ribose concentrations were determined by the method of Mejbaum [10]. Glucose was determined by the method of Morris [11].

After 8 hours of incubation, to reduce the carbohydrate content, the mixture was heated in a boiling-water bath. Trypticase (5 per cent), peptone (0.5 per cent), and sodium thioglycollate (0.1 per cent) were added to the different solutions and filter-sterilized. Different solutions were dispersed in screw-capped tubes and inoculated with 10⁴ spores of type E per tube. Each tube was overlaid with sterile vaspar, cooled in ice-water, and incubated at 60°F (15.6°C). The tubes were examined daily for gas production and assayed for botulinum toxin after 11 days of incubation.

2.8.2. Results

After 8 hours of incubation at 25°C (77°F), the particulate enzyme system had reduced the glucose concentration by 75 per cent and the ribose concentration by approximately 90 per cent.

TABLE IV. OUTGROWTH AND TOXIN PRODUCTION OF *Cl. botulinum* TYPE-E SPORES AT 60°F (15.6°C) IN TRYPTICASE-PEPTONE MEDIUM WITH THE CARBOHYDRATES REDUCED BY PARTICULATE ENZYME SYSTEMS

Treatment	Incubation time before gas production	Toxin titre after 11 days	
		Untrypsinized	Trypsinized
		(MLD/ml)	(MLD/ml)
TP + glucose	5	200	40 000
TP + glucose enzyme treated	7	10	2 000
TP + ribose	5	> 100	> 2 000
TP + ribose enzyme treated	6	10	1 000

The results of the outgrowth and toxigenesis of type-E spores in enzyme-treated and untreated glucose- and ribose-containing mixtures is summarized in Table IV. Gas production was observed after 5 days in the untreated glucose solution and after 7 days in the enzyme-treated solutions. There was a marked difference in the toxin titre of these mixtures after 11 days of storage at 60°F (15.6°C). The toxin titres of the untreated glucose mixture were 40 000 MLD/ml. In contrast, the toxin titre of the enzyme-treated solutions was 2 000 MLD/ml. A similar trend was observed in ribose solutions.

The results from these experiments indicate that the reduction of carbohydrate concentrations has a marked effect upon type-E toxin production.

Additional experiments are planned in laboratory medium (TP basal medium) and in fish substrates.

3. CHARACTERISTICS OF NON-PROTEOLYTIC *Cl. botulinum* TYPES B AND F AND THEIR POSSIBLE IMPORTANCE IN FISHERY PRODUCTS PRESERVED BY IONIZING RADIATION

Dolman et al. [12] reported that a non-proteolytic type-B strain isolated in their laboratory from a human botulism outbreak resembled type E in that it would grow and produce gas in cooked-meat medium after 42 days at 6°C (42.8°F).

In 1965, we reported the presence of non-proteolytic types B and F in marine sediments from the Pacific Coast of the United States of America. Subsequent studies of these new isolates have shown that their biochemical and physiological characteristics are indistinguishable from those characteristics possessed by type-E strains. The only difference among the B, E, and F cultures is the type of toxin produced. The non-proteolytic cultures of types B and F differ from the proteolytic strains in a number of characteristics. The characteristic of greatest importance in the preservation of fishery products by low doses of ionizing radiation is the ability of non-proteolytic types B and F spores to grow and produce toxin at low temperatures.

TABLE V. OUTGROWTH AND TOXIN PRODUCTION BY NON-PROTEOLYTIC TYPE F AT DIFFERENT TEMPERATURES IN CM-MEDIUM (2×10^6 spores/tube)

Outgrowth and toxin production	Incubation temperature			
	50°F	42°F	38°F	36°F
Earliest outgrowth ^a	5	15	39	> 102
Toxin assay MLD/ml	2000 (22 days)	100 (26 days)	20 (52 days)	< 4 (102 days)

^a Incubation time before visible growth or gas production.

Table V summarizes the outgrowth time and toxin production by non-proteolytic type-F spores at different temperatures in cooked-meat medium. In this medium, the spores can germinate, grow, and produce toxin at temperatures as low as 38°F (3.3°C). None of the cultures produced gas or detectable amounts of toxin at 36°F (2.2°C) during the 102-day incubation period.

Table VI summarizes the outgrowth time and toxin production by different concentrations of non-proteolytic type-F spores at 42°F (5.6°C) in cooked-meat media. Outgrowth and toxin production occurred even with spore inocula of only 10 spores per tube.

The outgrowth time of type-B spores at 42 and 38°F (5.6 and 3.3°C) was somewhat slower than that of type-F strains (see Table VII). However, toxin titres were usually higher with the type-B cultures at comparable incubation periods. The results from experiments with different spore concentrations are summarized in Table VIII. Outgrowth and toxin production was observed for each of the spore concentrations. After 64 days of incubation at 42°F (5.6°C) the toxin titre was 2000 MLD/ml irrespective of inoculum level.

Based upon these data, the outgrowth time of the non-proteolytic type-F strain in the temperature range of 38 to 42°F (3.3 to 5.6°C) is comparable with that of type E reported by Schmidt et al. [3]. The type-B cultures required a longer incubation period for outgrowth than either type-F or -E spores. However, toxin titres were generally higher for the type-B cultures.

Inoculated-pack studies are planned with non-proteolytic type-B and -F spores. From these data, we will determine whether the type-B and -F strains could be of any greater public health significance at temperatures lower than type E.

TABLE VI. OUTGROWTH TIME AND TOXIN PRODUCTION BY DIFFERENT CONCENTRATIONS OF NON-PROTEOLYTIC TYPE-F SPORES IN CM-MEDIUM INCUBATED AT 42°F (5.6°C)

Spores/tube	Earliest outgrowth ^a	Toxin titre ^b
	(Days)	(MLD/ml)
10 ⁶	15	100
10 ⁵	16	100
10 ⁴	20	100
10 ³	21	200
10 ²	25	200
10 ¹	26	200

^a Incubation time before visible growth or gas production.

^b Assayed after 64 days in trypsinized state.

TABLE VII. OUTGROWTH AND TOXIN PRODUCTION OF NON-PROTEOLYTIC TYPE B AT DIFFERENT TEMPERATURES ON CM-MEDIUM (2×10^6 spores/tube)

Outgrowth and toxin production	Incubation temperature			
	50°F	42°F	38°F	36°F
Earliest outgrowth ^a	5	17	> 109	> 120
Toxin assay MLD/ml ^b	20 000 (22 days)	200 (21 days)	20 (90 days)	< 4 (120 days)

^a Incubation time before visible growth or gas production.

^b Assayed I.P. in trypsinized state.

TABLE VIII. OUTGROWTH TIME AND TOXIN PRODUCTION OF DIFFERENT CONCENTRATIONS OF NON-PROTEOLYTIC TYPE-B SPORES IN CM-MEDIUM INCUBATED AT 42°F (5.6°C)

Spores/tube	Earliest outgrowth ^a	Toxin titre ^b
	(Days)	(MLD/ml)
10^6	15	2000
10^5	17	2000
10^4	19	2000
10^3	25	2000
10^2	26	2000
10^1	30	2000

^a Incubation time before visible growth or gas production.

^b Assayed after 64 days in trypsinized state.

4. BACTERIOPHAGES OF THE DIFFERENT TYPES OF Cl. botulinum

In recent surveys of the incidence of Cl. botulinum in soil, marine and freshwater sediments, etc., non-toxicogenic organisms with characteristics identical to Cl. botulinum have frequently been observed. Pure cultures of Cl. botulinum also have been reported to revert to the non-toxicogenic state. The factors that are responsible for the toxigenicity of such cultures have not been identified.

With certain micro-organisms, the bacteriophages play a very important role in the conversion of non-toxicogenic organisms to the toxicogenic form. It

was reported by Freeman [13] that exposure of certain non-toxigenic strains of Corynebacterium diphtheriae to specific bacteriophages resulted in the appearance of toxigenic C. diphtheriae.

Much of our effort during the past year was devoted therefore to studying the relationship of bacteriophages of Cl. botulinum to the toxigenicity of the bacterium, bearing in mind that an understanding of the factors responsible for the toxigenic characteristic may very well give us an insight into how to control toxin production.

The first phase of these studies was to determine whether the different types of toxigenic Cl. botulinum were lysogenic. In this work, mitomycin-C was used to activate the prophage and induce lysis of the bacterial cells. Mitomycin-C was used at concentrations of 0.1 to 5.0 $\mu\text{g/ml}$ to induce lysis. The effect of the various concentrations of mitomycin-C on the growth of different types of Cl. botulinum are summarized in Fig. 16.

Lysis occurred in all of the strains treated with mitomycin-C except the non-toxigenic cultures of 066 BNT (a non-toxigenic variant from toxigenic strain 066B isolated by Don Kautter, Food and Drug Administration).

The concentration of mitomycin-C yielding maximal lysis was 1 $\mu\text{g/ml}$ for type A, non-proteolytic and proteolytic types B, non-toxigenic organism (strain P4) resembling type E, and proteolytic type F. Maximal lysis was obtained with type-E cultures at 0.1 and 0.5 $\mu\text{g/ml}$ and with non-proteolytic type F at 0.5 and 1.0 $\mu\text{g/ml}$. All of the toxigenic cultures tested thus far have been lysogenic.

Bacteriophages and phage tail-like structures were observed in the lysates of the different types of Cl. botulinum. These phages are classified into four groups.

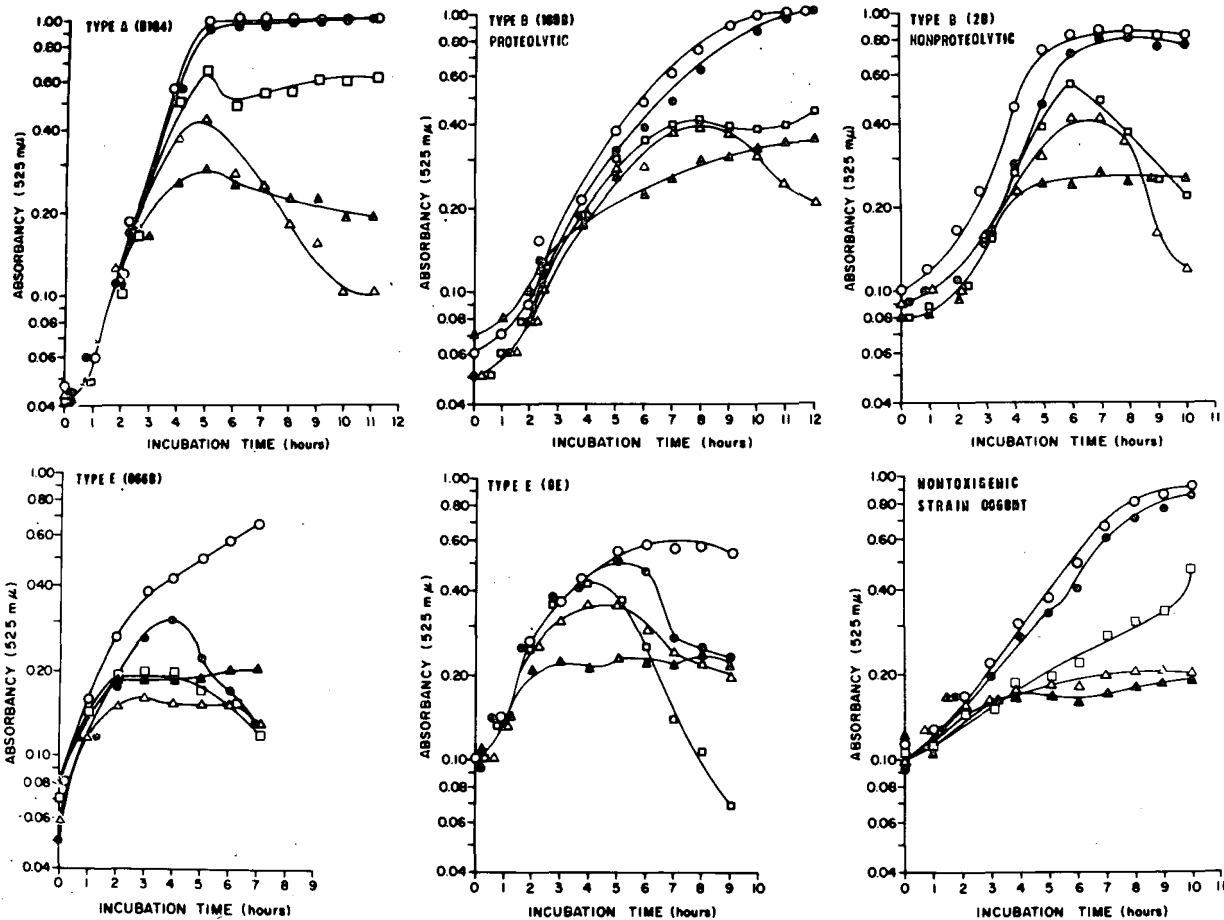
The first group consists of phages from Cl. botulinum type A, proteolytic and non-proteolytic types B, and certain strains of type E (Beluga, 92E, and 066B). These phages have long flexible tails and hexagonal heads.

The second group consists of non-proteolytic type-F phages. Two different phages were observed in lysates of non-proteolytic type F. One of the phages is similar to phages of group one in that it has a hexagonal head and long flexible tails. The other phage also exhibits a hexagonal head and a tail surrounded by a contracted sheath.

The third group consists of proteolytic type-F phages. This phage exhibits an elongated head and a long tail.

The fourth group consists of phage tail-like structures. These phage tail-like structures were observed in lysates of non-toxigenic E-like organisms and the prototype type-E (8E strain) culture. These studies are similar to those reported by Inoue and Iida [14].

The second phase of these studies was to search for indicator strains for the different phages. A plating system has been developed to detect phage plaques on bacterial lawns. Phages of non-proteolytic types B and F and proteolytic type F have been propagated. Determinations of the relationship of these phages to the toxigenesis of Cl. botulinum is currently in progress.



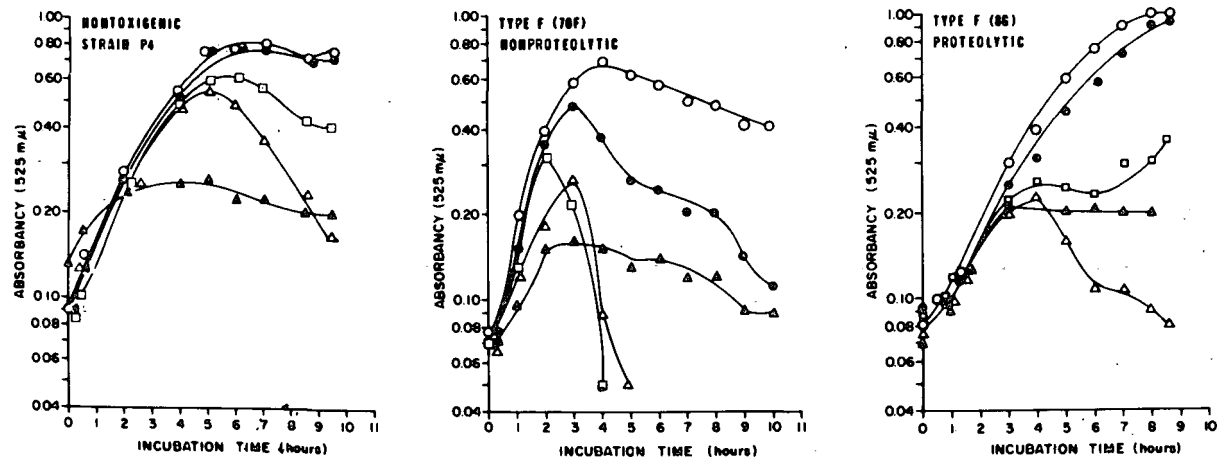


FIG. 16. Effect of mitomycin C concentrations on growth of different types of *Cl. botulinum* at 25°C: control, O; 5 μg/ml, ▲; 1 μg/ml, △; 0.5 μg/ml, □; and 0.1 μg/ml, ⊙.

ACKNOWLEDGEMENTS

These investigations were supported by the Division of Isotopes Development and Division of Biology and Medicine of the U.S. Atomic Energy Commission.

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DOSIMETRY AS A FACTOR IN ASSESSING THE TECHNICAL EFFECTS OF IRRADIATION ON FOOD PRODUCTS

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Abstract

DOSIMETRY AS A FACTOR IN ASSESSING THE TECHNICAL EFFECTS OF IRRADIATION ON FOOD PRODUCTS.

The importance and need of universal uniform methods of measuring and reporting absorbed doses as related to the interpretation of irradiation effects on food items are discussed. The need and importance for uniformity of dose in the interpretation of microbiological and organoleptic studies of fishery products are specifically discussed using limited actual data as well as an illustrative example.

In reviewing results of post-irradiation studies on food products, one occasionally finds disagreement in results. These differences may be as wide apart as the statute miles that separate the investigators. Other times, the differences may be minuscule so that one tends to dismiss these differences as insignificant.

Many rationales have been set forth to explain these differences, such as possible differences in maturity, freshness, native harvest conditions, climate, etc. There is no doubt that these factors do exert some influence on the assessment of total post-irradiation effect. One other factor that the Atomic Energy Commission (AEC) has been concerned with, which up until July 1969 [1] had not been to this writer's knowledge openly questioned, is what influence does the uniformity of dose exert on the interpretation of post-irradiation storage effects? The exactness of this variable is of most importance in interpreting results not only within a laboratory, but between investigating groups anywhere in the world.

If one were asked for an interpretation of the significance of the following statements:

"Uninoculated fillets exposed to 100 krad were considered spoiled by the entire test panel after 28 days of post-irradiation storage at 45°F. Fillets receiving the same dose inoculated prior to irradiation with 10⁴ spores of Clostridium botulinum, Type E, showed toxin development after 45 days under similar storage conditions".

Most assuredly, the majority would answer, "It is self-explanatory."

In a recent survey of AEC contractors, few could agree on a correct method of measuring the absorbed dose delivered in a food irradiator and on

how to interpret the dosimetry data. Some of the questions which were raised were: Should we be measuring maximum doses, minimum doses or average doses? Which dosimeters should be used? How many? Where should they be placed? How should the values be used?

These are just a few of the factors that must be considered in establishing the dose levels received by the irradiated product. That such considerations are deemed important is also recognized by the proposed petition regulations issued by the U.S. Food and Drug Administration (FDA) in 1967 [2]. Prior to this issuance, a seminar was held in May 1967 by the FDA. The following is a quote from that seminar on the subject of dosimetry and related effects:

"Radiation dosimetry is important in evaluating all the effects of the radiation. Before the effects can be evaluated adequately, the petition must supply complete details of the source energy and strength, uniformity of the radiation field, and depth-dose distribution within the irradiated media. Whenever the petition purports to establish that a given dose has produced a given effect, all of the dosimetry details must be included. The measurement of the dose can be made during experimental studies by any convenient and accurate means, as long as the technique is described in detail and the accuracy of the measurement established. The petitioner must supply, however, the details of the direct dose measurements used in his reported experiments". [3]

Those who are working in the field of food irradiation must be able to explain to their own country's health authorities what is meant by the reported dose. The absolute minimum and maximum doses received by the target must be known in order to clearly interpret post-irradiation effects.

Irradiation processing seems to carry some mystique about it, however, it is not dissimilar to thermo-processing. Whenever one is exploring the thermal processing parameters for a new product, new size containers, etc., certain routine tests are set up. One series of tests of significant importance are those dealing with heat penetration. It is a well-known fact in thermo-processing that the size and shape of the container, the nature of the product, the type of thermo-process, and many other factors must be taken into consideration in determining processing parameters. It is also appreciated that once a variable is changed, e.g. container size, new data must be collected to insure proper processing. Irradiation processing is no different. Energy is absorbed by the target and certain effects take place. The rate of energy deposition is measured in thermo-processing by means of thermocouples properly placed in the "coldest" spot (which will depend upon the product and geometry) and at the "hottest" spot. Process time is based upon the time required for the "cold spot" to reach the desired process temperature.

In irradiation processing the energy absorbed is measured with a dosimeter and, as in heat processing, it is a function of the strength of the source and the time duration of exposure. There will always be an absorbed-dose gradient from the outside to the inside of a target. This gradient must be determined and maintained, if the process is to be effective. Within the limits

of experimental error, the investigator must know the absolute minimum and maximum doses for each specific target geometry. Yet, all too frequently, in published reports of food-irradiation investigations, the experimenter has failed to fulfil this requirement.

In the USAEC, DID, Food Irradiation Program it has been necessary in many instances to perform "after-the-fact" recalibration of irradiation facilities to salvage costly biological studies in which the dosimetry was neglected. Presently, a dosimetry manual is in preparation at the Brookhaven National Laboratory [4] which describes in detail how to characterize and calibrate any type of irradiator. It deals with methods of how to find and measure the true minimum and maximum points in an irradiator target. This manual will be available shortly for dissemination to the public.

In the case of marine-product irradiation the uniformity of dose is as important in assessing the spoilage time of fillets (X-values) as it is in the assessment of potential Cl. botulinum toxin outgrowth.

In both evaluations, the end-point is a function of microbial survival which is dose dependent. In the X-value studies, spoilage is determined by odour alone. This odour development is a function of the microbial flora surviving the irradiation treatment. If there is a large spread between the minimum and maximum doses, the results for any one sitting can fluctuate more widely than is desirable. Those panelists receiving samples irradiated at the minimum will report spoilage earlier than panelists receiving samples irradiated at the maximum. This could influence the day of total olfactory rejection (DOTOR).

Assessment of the Cl. botulinum problem in irradiated fishery products is being measured by the inoculated-pack technique. As with odour development, the possible outgrowth of toxin is markedly influenced by the level of microbial organisms surviving irradiation.

Again it is necessary that experimental samples be irradiated uniformly if the results for a given dose level are to be clearly defined.

The X-values and inoculated-pack studies have been predicated upon the fact that toxin development in unirradiated fillets is inhibited by the metabolic products of the spoilage organisms plus the safety factor that unirradiated fish spoils at a rate much faster than the outgrowth of botulinum spores. Those receiving the minimum dose will tend to spoil faster while inhibiting the outgrowth of toxin, where the reverse might be true for the samples receiving the maximum dose. Hence, the more uniform the dose for any given treatment level, the less fluctuation and anomalies will be seen. The true significance of the application of irradiation can then be assessed.

Results have been presented in the open literature to indicate that at certain storage temperatures samples inoculated with large spore levels which had been irradiated at the "200-krad" level might present a potential botulinum problem. This is rarely seen at similarly treated fillets irradiated at the "100-krad level". Without adequate dosimetry these results are hard to assess. What is needed is knowledge of the absolute minimum and maximum doses. It is not impossible to provide uniformity ratios close to 1.0. From Fig. 1, a uni-

formity ratio for Lot A using the plane in which dosimeters 8—12, 18—22, and 30—34 lie, is calculated from actual data to be 1.13. In addition to providing a minimum uniformity ratio, a narrow range between dose-exposure levels is required so that a dose-response curve might be constructed and superimposed on a spoilage curve. Using the most severe conditions of the inoculated-pack studies and using fillets of prime quality for the spoilage

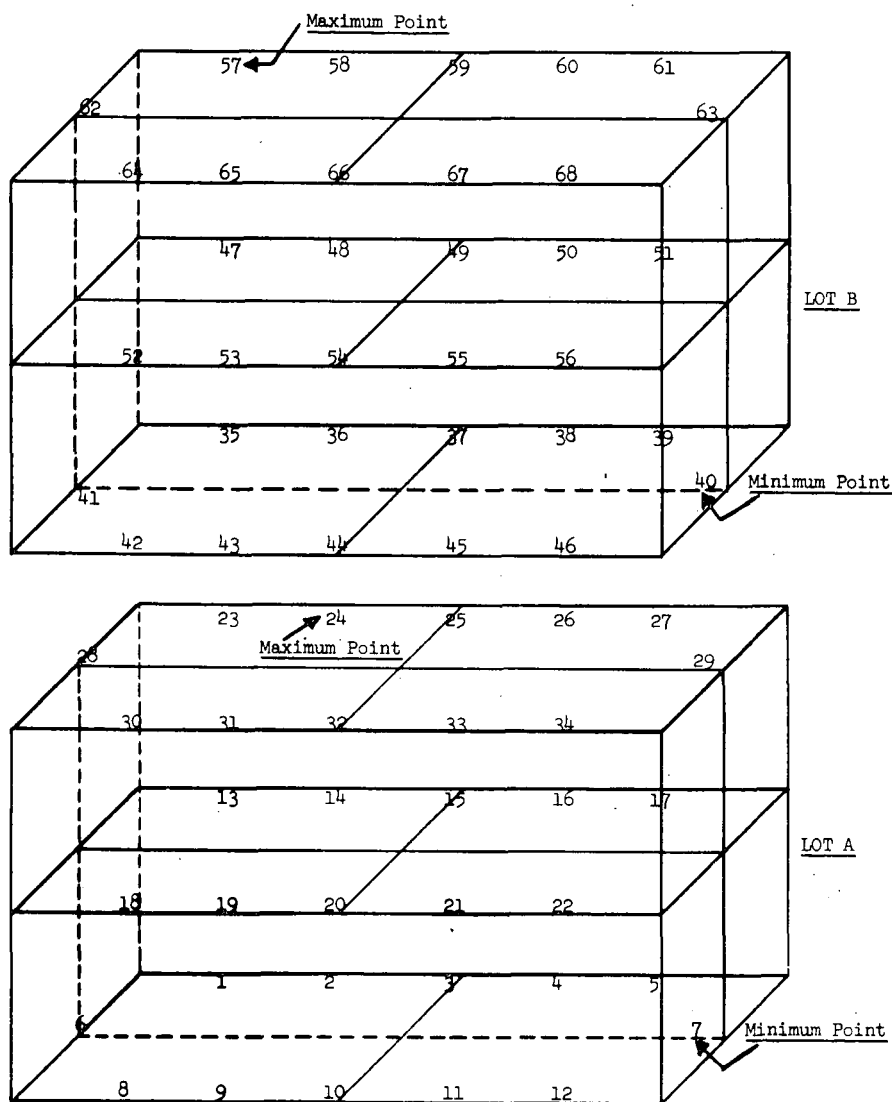


FIG. 1 Arrangement of the ferrous-cupric sulphate dosimeters and positions of the minimum and maximum dose points in series 1 of the inoculated-pack studies.

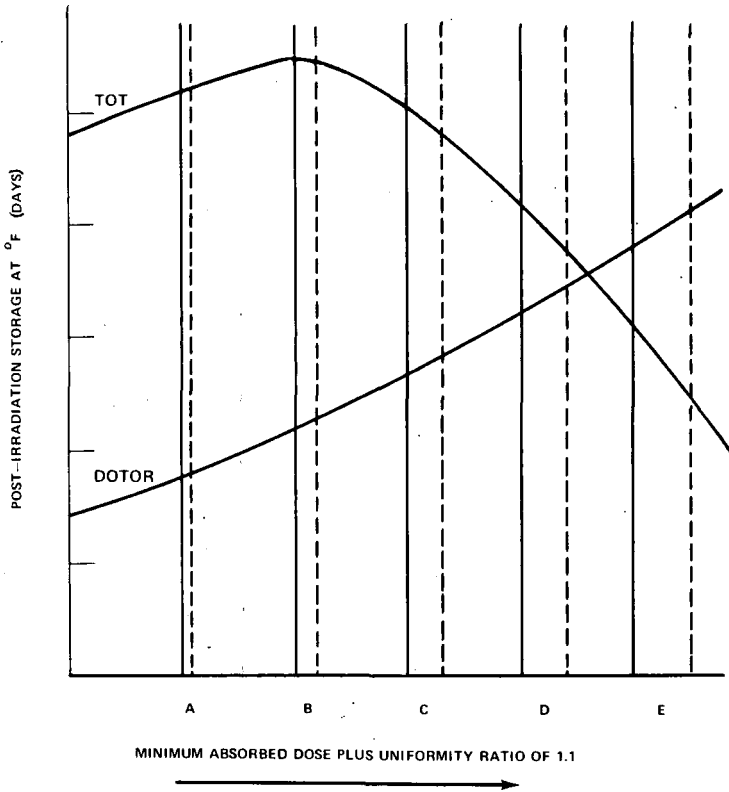


FIG. 2 Illustrative summary data curve comparing time of toxin development (TOT) with spoilage time (DOTOR)^a.

studies, the potential of a public health hazard could be more accurately assessed relative to dose. An over-simplified illustrative example of what such a curve might look like is shown in Fig. 2.

This figure would be drawn from data collected using the most severe conditions of the tests, i.e. heaviest spore concentration inoculated into prime-quality fillets. The question of any Cl. botulinum problem in irradiated fishery products could be more accurately assessed. This illustrative curve suggests that, under the severe conditions of the tests, the maximum dose level at which a regulation should be issued would be no higher than the minimum level of dose "D".

In comparison to the above concept, the following example is presented based upon actual data. Two experiments were conducted for the inoculated-pack studies where two different target geometries were used. In series 1

^a DOTOR = Day Of Total Olfactory Rejection.

(Fig. 1) both the "100-krad" and "200-krad" variables were irradiated at separate times. The minimum and maximum points are indicated. Lot A represents the "100-krad" variable and Lot B the "200-krad" variable. In series 2, the samples representing the higher dose were placed in the bottom of the irradiation chamber and those representing the lower dose on top of that. The samples were lowered to the source and exposed to the time calculated to give the lower dose. The chamber was raised, the "100-krad" variable removed, and the chamber again lowered for an additional "100-krad" exposure. Figure 3

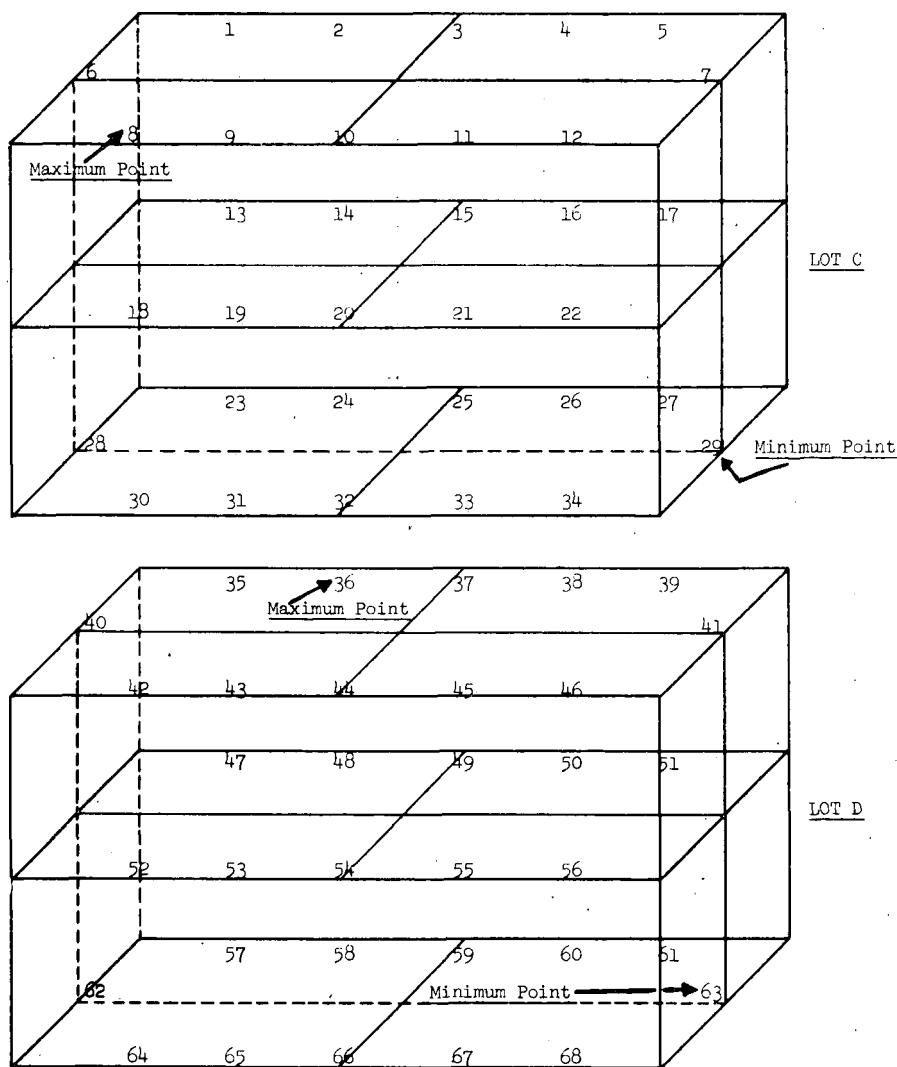


FIG. 3 Arrangement of the ferrous-cupric sulphate dosimeters and positions of the minimum and maximum dose points in series 2 of the inoculated-pack studies.

shows the position of the minimum and maximum points for both lots. Lot C represents the "100-krad variable" and Lot D the "200-krad variable". Notice the shifting of the maximum points between the two lots due to the changing of target geometries.

Both of these series had different target geometries and results of the inoculated packs might be askew based on improper exposure practices. Reconstruction "after-the-fact" dosimetry provided the following uniformity ratios:

- (a) Series 1, Lot A = 1.93 (145 krad/75 krad)
- (b) Series 1, Lot B = 2.00 (300 krad/150 krad)
- (c) Series 2, Lot C = 1.79 (140 krad/78 krad)
- (d) Series 2, Lot D = 1.83 (257 krad/140 krad)

Review of the inoculated-pack studies, with this new information, is currently in progress. It is expected that when this review is completed a better interpretation of the past data will result. Newly initiated inoculated-pack studies will be conducted using uniform target geometries which provide the most uniform dose to clearly define the results for a given dose level.

In summary, it cannot be stressed strongly enough that adequate and uniform dosimetry practices must be considered in the conduct of all experiments concerning the irradiation of food products.

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¹ A working draft for limited distribution only.

SUMMARIES, CONCLUSIONS AND RECOMMENDATIONS OF THE PANEL

The usefulness of ionizing radiation as a food-processing method has been amply demonstrated for a variety of food products. On a world basis the radiation processing of foods has progressed to the stage that approval has been given in certain countries for the manufacture and unlimited consumption of individual types of food, such as: potatoes and onions irradiated for sprout inhibition; wheat and wheat products, dried fruits and dry food concentrates irradiated for insect control; and mushrooms irradiated to extend the market life.

Since food products derived from the sea and fresh-water sources are high in protein content and are available in varying amounts in most countries of the world, it is logical that preservation of these products by radiation should be considered as one means of increasing the quantities of protein-rich foods for certain populations of the world. This, in addition to the possible use in the present normal distribution of fresh and semi-processed fishery products.

Although extensive work has been done on fresh fish, shrimps, crab, lobsters and other types of fishery products, it was realized that more work would be necessary to resolve many problems of the irradiation of such products before a successful commercial process can be derived and public health clearances for consumption obtained in the various countries. Therefore, a Panel was convened in Vienna, Austria, from 15 to 19 December 1969, to go over the informational material available and to suggest future programs.

The work of the Panel, under the chairmanship of Dr. H.E. Goresline, was designed to review the results of research, to discuss the problems that have been recognized and to give advice in the following areas:

1. Market-life extension.
2. Standardization of working tools.
3. Clostridium botulinum as a factor in wholesomeness of fishery products.

After having studied and discussed the technical papers presented by Panel Members, as well as specific questions raised by the Secretariat, and having drawn upon their personal experiences in research on the irradiation of fishery products, the Panel came to the following conclusions:

1. MARKET-LIFE EXTENSION

There exists a need for the extension of market life of various fishery products to elevate the nutritional and economic status of the population in various countries. World-wide reports have consistently indicated that various

fishery products can be treated with low doses of radiation to extend their market life. Notable differences in market-life extensions for the same products have been reported by different investigators. Discussion as to the methods of clarifying and rationalizing these differences was a subject covered by this Panel.

2. STANDARDIZATION OF WORKING TOOLS

Presently, in the field of food irradiation there is little standardization regarding the measurement and expression of either the radiation dose or the product quality.

3. Cl. botulinum AS A FACTOR IN THE WHOLESOMENESS OF FISHERY PRODUCTS

Radurization of fresh fishery products is a feasible process if the final products are stored below 3°C, achieving a two or three times increase in shelf life with no known health hazard. Botulism results from the consumption of food containing toxin. For a food to become toxic, firstly the causative organism must be present and secondly, the food must be exposed to conditions that will support the spore outgrowth and toxin production. There is sufficient evidence from the surveys carried out in different parts of the world on fresh fish, samples of various bodies of water and bottom deposits, to conclude that any preservation process for fishery products must assume the presence of Cl. botulinum. Marine products vary markedly as a substrate for supporting the growth and toxin production of Cl. botulinum, type E. The time-temperature relationships for growth and toxin production vary with the product. Therefore, each product must be assessed as to its ability to support toxin production.

When refrigeration is an integral part of the processing and distribution system, a potential hazard exists if the non-proteolytic Cl. botulinum, types B, E and F are present. The minimum reported temperature at which these non-proteolytic types will grow and produce is 3.3°C. Where no refrigeration of the product is required, then all types of Cl. botulinum must be considered. The evidence available to the Panel, from the results of irradiated experimental inoculated packs, indicates that when fishery products are radurized and stored above 3.3°C, there can be an increase in the botulinum hazard. The evidence is not precise enough to establish the extent of this increase. The possibility of such an increased hazard is not unique to the radurization process.

RECOMMENDATIONS:

Having assessed the problem areas in the irradiation of fishery products and discussed the need for additional information and additional tools with which to work, the Panel made the following recommendations:

1. MARKET-LIFE EXTENSION

- (a) Only fishery products of good quality should be irradiated for the purpose of prolonging their market life.
- (b) Market life must be defined in each area in order to take into account a variety of local conditions and preferences. When citing market-life studies or other periods of storage, the criteria on which the assessments were based should be expressed so that the results may be universally understood.
- (c) Strict control must be maintained with regard to the temperatures used in the handling and refrigerated storage of irradiated fishery products, and there must be no slackening in the enforcement of these restrictions. Therefore, the Panel recommended that a visual temperature indicator be developed for packages of products in order to be able to determine if the required temperatures have been adhered to.
- (d) If the distribution chain requires that the product be packaged, then the effect of the packaging material must be included in assessment of market life.
- (e) Since the chemical tests in general use to determine the market life of irradiated fishery products have been of limited value, the Panel recommends that more research be encouraged to find more satisfactory indices.
- (f) In view of the fact that total bacterial counts, as presently determined, have been of limited value in the determination of the market life of irradiated products, the Panel recommended that research be oriented towards the development of materials and methods suitable for the rapid assessment of microbial spoilage indicators.

2. STANDARDIZATION OF WORKING TOOLS

- (a) The dose distribution in each particular product and for each target geometry must be measured in order to determine the absolute minimum and maximum absorbed doses.
- (b) In order to avoid confusion, the measured minimum and maximum doses must be reported in an appropriate way, with expressions of statistical error (e.g. minimum dose and uniformity ratio and statistical variations, or both minimum and maximum dose values and statistical variation).
- (c) The dosimetry method used, and the calibration data for the source, must be described in detail and equilibrated to an internationally accepted standard (e.g. American Society for Testing and Materials). The dose distribution must be re-determined any time there is a change in the critical aspects of the operation (e.g. product density, package size, source replenishment, etc.).
- (d) The Panel recommended that there be continued research to find more practical and more convenient dosimeters for food irradiation.

- (e) The Panel agreed that standardization of sensory assessment methods in all laboratories is not practicable. It is recommended, therefore, that whatever system is used, the results should be treated in the appropriate statistical manner and full details presented. In addition, sufficient details of the sensory assessment method used should be presented to enable others to relate the results to other methods.
- (f) Market life must be defined in each country and the criteria must be so defined that results may be universally understood.
- (g) The Panel recommended that research be conducted to investigate physical and/or chemical indices of quality in irradiated fishery products (e.g. identification of residual bacterial enzymes and conductivity measurements).

3. Cl. botulinum AS A FACTOR IN THE WHOLESOMENESS OF FISHERY PRODUCTS

- (a) The Panel endorses the recommendations of the Panel on the "Elimination of Harmful Organisms from Food and Feed by Irradiation" (STI/PUB/200, IAEA, Vienna, 1968), that large-scale radurization experiments should be carried out on fish known to be naturally contaminated with Cl. botulinum, to aid in the assessment of the extent of the botulinum hazard.
- (b) More inoculated-pack studies should be done on various products at radurization doses in order to determine more precisely the conditions under which toxin may be produced. In addition, these studies should be extended to include a wide variety of fishery products.
- (c) Investigations should be carried out to improve the existing methods and to develop new methods for determining the degree of contamination of fishery products with Cl. botulinum.
- (d) The Panel strongly recommends the use of standardized microbiological procedures and sampling plans, in order to compare the results of the laboratories in various parts of the world.
- (e) Quantitative studies should be carried out to determine the natural incidence of Cl. botulinum in conventional fish-processing factories, in order to recognize the degree of contamination.
- (f) Since the concentration of type-E spores varies widely in nature, and certain areas appear to be free of them, studies of the factors leading to the development and accumulations of such Cl. botulinum are recommended.

The IAEA and FAO were requested by the Panel to sponsor research in the fields mentioned above.

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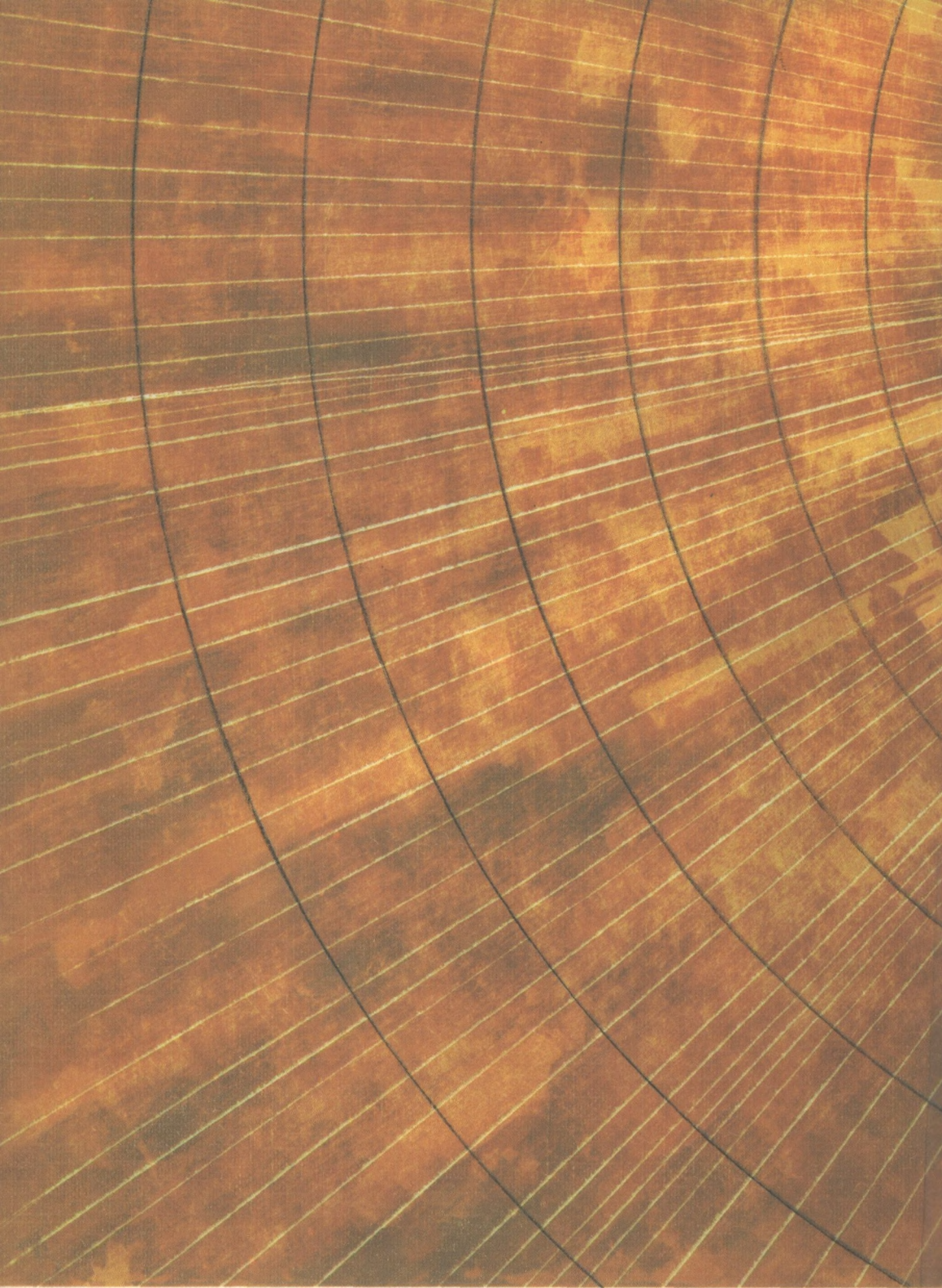
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VIENNA, 1970

PRICE: US \$5.00

Austrian Schillings 130,-
[£2.1.8 [£2.08]; F.Fr. 27,80; DM 18,30]

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