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IRRADIATION PRESERVATION OF FRESH - WATER FISH AND INLAND FRUITS AND VEGETABLES

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SUMMARY OF RESULTS

Results obtained during this second year of contract effort continue to indicate that irradiation pasteurization is able to significantly extend the refrigerated storage life of fresh-water fish as well as inland fruits and vegetables. The following quotations, taken from this report, illustrate these statements and other pertinent findings:

Yellow Perch:

Tests were conducted to confirm previous estimations of the extension of refrigerated storage life of yellow perch fillets. For fillets held at 33°F, the extension of storage life at 0.3 and 0.6 megarad was approximately 4- and 5-fold, respectively. Holding fillets at 42°F decreased the above maximum storage times by approximately 50 percent. It may be concluded that yellow perch fillets are amenable to the irradiation pasteurization process. Significant extension of storage life at 33°F can be attained at a dosage level of 0.3 megarad with only little detriment to initial quality.

Strawberries:

Gamma irradiation (200 krad) of strawberries, var. Robinson, will delay by 2-3 days the rapid decay caused by transportation without refrigeration and subsequent storage at 44°F.

Prunes:

The dehydration of prunes is facilitated by gamma irradiation (200-400 krad).

Potatoes:

The sprouting of Arenac and Ontario potatoes was inhibited by 7.5 krad of gamma radiation.

FOREWORD

For this effort, The University of Michigan provides irradiation services and general administration of the contracts through the Institute of Science and Technology. This is an arrangement of convenience. Each subcontractor, namely the Bureau of Commerical Fisheries and Michigan State University, is independently responsible for the scientific aspects of its respective research effort.

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INTRODUCTION

Irradiation pasteurization continues to demonstrate potential usefulness for preservation of fresh-water fish and inland fruits and vegetables. This is being demonstrated with fish caught in the Great Lakes and with fruits and vegetables grown in the State of Michigan. The fish are being evaluated by scientists at the U. S. Bureau of Commercial Fisheries Laboratories (BCF) in Ann Arbor, while the fruits and vegetables are being studied in the Department of Food Science at Michigan State University (MSU). Most of the irradiations are carried out in the cobalt-60 gamma ray sources belonging to The University of Michigan (UM), although a resonance transformer at MSU is also used occasionally for this purpose.

The degree of success attained by various treatments for preserving foods is evaluated by organoleptic, chemical and microbiological tests. Through the present contract effort, both equipment and personnel have been added which materially enhance the capacity of these research groups to test and evaluate irradiated foods. Furthermore, the cobalt-60 sources at the UM have been enlarged and equipped for irradiation at refrigeration temperatures.

Since the work is being carried out by three different groups, this report accordingly consists of three sections, each section having been prepared by the group concerned.

Section 1

IRRADIATION PRESERVATION OF FRESH-WATER FISH

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SUMMARY

The current contract, Subcontract No. 1 under Prime Contract No. AT(11-1)-1283, was initiated April 15, 1963, between the Bureau of Commercial Fisheries Technological Laboratory, Ann Arbor, Michigan, and the Institute of Science and Technology, The University of Michigan. The objective of the study is to investigate the application of cobalt-60 gamma irradiation at sub-sterilization doses to the extension of the refrigerated shelf life of the two most commercially significant Great Lakes fishery products, fresh yellow perch fillets and smoked chub, and the potential usefulness of gamma radiation for controlling the activity of enzymatic antimetabolites of fish and fishery products.

Tests were conducted to confirm previous estimations of the extension of refrigerated storage life of yellow perch fillets. For fillets held at 33°F, the extension of storage life at 0.3 and 0.6 megarad was approximately 4- and 5-fold, respectively. Holding fillets at 42°F decreased the above maximum storage times by approximately 50 percent. It may be concluded that yellow perch fillets are amenable to the irradiation pasteurization process. Significant extension of storage life at 33°F can be attained at a dosage level of 0.3 megarad with only little detriment to initial quality.

Microbiological studies were conducted concurrently on the above products. The initial microbial load on commercially hand-filleted yellow perch fillets, which usually was between 10^5 and 10^6 , was reduced approximately 99 percent by 0.3 megarad and nearly 100 percent by 0.6 megarad of gamma irradiation at 33°F. The microbial lag period was increased by radiation. The degree of increase was a function of dosage; longer lag periods were obtained at the higher radiation dose. Increases in dosage did not decrease the growth rate of the microbial population.

Studies on the generic identification of bacteria were conducted and the methodology is discussed. Organisms found in the nonirradiated perch on initial isolation in order of decreasing number were: Pseudomonads, Micrococcus, Brevibacterium, Achromobacter, Alcaligenes, Bacillus, Sarcina, Corynebacterium, Flavobacterium, and a few Proteus, Escherichia, and Lactobacillus. As spoilage progressed, the pseudomonads became increasingly predominant.

Irradiation of samples to 0.3 and 0.6 megarad eliminated the pseudomonads. The residual flora during storage was primarily comprised of the achromobacter-alcaligenes group.

A suitable experimental substrate for the study of microbial proteolytic activity during fish spoilage is discussed. Proteolytic activity was demonstrated by some of the pseudomonads, brevibacteria, micrococci, sarcina, corynebacteria, and yeasts. Irradiation eliminated essentially all proteolytic bacteria.

Procedures used for the conservation of bacteria through the utilization of lyophilization are presented.

Studies were conducted to determine the effect of irradiating temperature on the irradiation flavor development in smoked chub. It was found that the irradiation of raw chub at 0°F, as against 33°F, significantly reduced irradiation flavors imparted to the product.

Whole fish samples were irradiated at various dosage levels under various conditions of temperature and pH, to determine the effect of these variables on the radiosensitivity of thiaminase in situ. Radiation had no apparent effect on the enzyme when the samples were irradiated in the frozen state. At temperatures above freezing, thiaminase was destroyed by irradiation. Thiaminase appeared to be least stable at the natural pH of the fish tissue. There appeared to be slight differences in the radiosensitivity of the thiaminase of different species.

INTRODUCTION

This annual report represents the second year of effort in accordance with the terms of Subcontract Number 1 under Prime Contract Number AT(11-1)-1283, entitled "Irradiation Preservation of Freshwater Fish" initiated April 15, 1964, between the Bureau of Commercial Fisheries Technological Laboratory, Ann Arbor, Michigan and the Institute of Science and Technology, The University of Michigan, Ann Arbor. The overall objectives of the study are to investigate the application of cobalt-60 gamma irradiation at substerilization doses to the extension of the refrigerated shelf life of the two most commercially significant Great Lakes fishery products; fresh fillets of yellow perch (Perca flavescens) and smoked chub (Coregonus hoyi), and to determine the potential usefulness of gamma irradiation for controlling the activity of enzymatic antimetabolites of fish and fishery products. The conclusions made in this report are tentative as all phases of this study are continuing.

PART A. SHELF LIFE STUDIES

Yellow Perch

Re-Evaluation of Irradiated Yellow Perch Fillets Stored at 42°F

Objective

To confirm the results of a previous and similar test to estimate the shelf life of yellow perch fillets irradiated to 0.0, 0.3, and 0.6 megarad and stored at 42°F.

Materials

Thirty-five pounds of commercially hand-cut yellow perch fillets produced from Lake Michigan gillnetted fish (South Haven, Mich., April 1964).

Procedures

All procedures remained essentially the same as previously reported (Emerson et al., 1964) for the comparable test with the following exceptions: (1) sensory evaluation of odor was conducted on raw samples as well as cooked samples, (2) chemical evaluation was limited to TVA (total volatile acids) determinations.

Results and Discussion

Sensory Evaluation: Tables 1 and 2 present the sensory scores from odor evaluation of raw and cooked fillets, respectively. Little difference was noted between the results of the two methods of testing. Unirradiated fillets were unacceptable by the 6th day of storage. Fillets irradiated to 0.3 and 0.6 megarad were found to be unacceptable after 15 and 20 days, respectively.

Several problems in evaluation were encountered during the course of the test. Primarily, the panel members indicated difficulty in determining strict differences between reference and treatment samples without allowing hedonic considerations to influence their decisions. Also, the changes in odor that develop during storage in unirradiated samples are dissimilar from those which develop in irradiated samples. Thus, a change in odor of a certain magnitude in an unirradiated sample may give a grossly different indication of the degree of spoilage which has occurred in that sample than would an identical magnitude of change in an irradiated sample. In many instances the panel scored both the unirradiated sample and an irradiated sample the same, yet the panel considered (hedonically) the former spoiled and the latter still acceptable.

Table 1. —Sensory evaluation¹ of irradiated yellow perch fillets stored at 42°F — Raw fillets.

Days in Storage	Odor Scores (Raw)		
	0.0 Mr	0.3 Mr	0.6 Mr
2	10	6	3.7
6	3.7	8.3	7.0
8	--	7.7	6.0
10	--	5.3	5.0
15	--	6.8	6.0
20	--	5.0	6.4
24	--	--	3.3

¹Based on 9-point hedonic scale, where: 9=like extremely, 1=dislike extremely. A score of 5 or higher indicates acceptance of product.

Table 2. —Sensory evaluation of irradiated yellow perch fillets stored at 42°F — Cooked fillets.

Days in Storage	Odor Scores (Cooked)		
	0.0 Mr	0.3 Mr	0.6 Mr
2	8.6	6.7	8.3
6	3.3	7.3	7.7
8	--	7.0	6.7
10	--	8.0	5.0
15	--	5.0	6.7
20	--	4.7	4.4
24	--	--	5.0

I was fortunate to have an occasion to discuss this general problem with Dr. T. H. Simpson, Torrey Research Station, during his recent stay in this country. He provided a number of useful suggestions in regards to increasing the meaningfulness of our sensory evaluations. As a result, future evaluations of yellow perch fillets will be based entirely on the 9-point hedonic scale as the method of scoring, and the triangle method of sample presentation. After initial establishment of degree of preference between unirradiated versus irradiated samples, each individual triangulation shall be limited to one test variable — time in refrigerated storage. That is, unirradiated samples will be compared only to unirradiated references, and irradiated samples only to irradiated references.

Microbiological Examination: Table 3 illustrates the variations in total aerobic microbial plate counts, total yeasts and molds plate counts of the air-packed unirradiated and irradiated yellow perch fillets stored at 42°F. A preliminary report of the results obtained from the vacuum-packed samples (0.0 Mrad and 0.6 Mrad) is also included.

The initial microbial count (20°C) of the unirradiated air-packed samples was an average of approximately 1.86×10^5 and the psychrophilic count (3°C) was approximately 5.95×10^4 . By the 4th day of storage, the sample had reached a total count of approximately 1.68×10^8 at both incubation temperatures (20°C and 3°C). Similar values were obtained in the vacuum-packed samples. The yeasts and molds total counts increased only 10-fold during the 4 days of storage and only an insignificant increase was observed in the vacuum-packed samples.

Irradiation to 0.3 megarad reduced the total microbial count by approximately 99.2 percent. Initial unirradiated values in the air-packed samples were exceeded by the 5th day of storage and reached a total of 3.12×10^8 by the 14th day. Psychrophilic (3°C) total counts followed the same growth pattern. Unfortunately, vacuum-packed samples at this radiation level were omitted due to the limited sample capacity in the irradiator.

Irradiation to the 0.6 megarad level reduced the total microbial count by nearly 100 percent and the count progressively increased to reach a count of 3×10^8 by the 24th day of storage in the air-packed samples. By the 37th day of storage the vacuum-packed had only reached a count of 8.52×10^6 and at that time the test was terminated due to depletion of irradiated vacuum-packed samples.

Yeast and mold counts of the samples were reduced by both irradiation levels. At 0.3 megarad the counts remained at insignificant numbers and at 0.6 megarad the counts were reduced nearly 100 percent during the first 7 days of storage reaching then a count of 6.32×10^6 the 24th day of storage. The counts in the vacuum-packed samples did not increase as expected throughout subsequent storage.

Dose (Mr)	Storage time (days)	Air-Packed				Vacuum-Packed			
		Microbial counts		Yeasts and molds		Microbial counts		Yeasts and molds	
		per gram		per gram		per gram		per gram	
		20°C	30°C	20°C	30°C	20°C	30°C	20°C	30°C
0.0		1.68×10^5	5.60×10^4	0.96×10^3	0.12×10^3				
		1.48×10^5	5.92×10^4	1.56×10^3	0.12×10^3				
		2.44×10^5	6.32×10^4	0.96×10^3	0.36×10^3				
		av:							
	0 ¹	1.86×10^5	5.95×10^4	1.16×10^3	0.20×10^3				
						5.80×10^5	2.60×10^5	0.96×10^3	0.12×10^3
						4.08×10^5	4.28×10^5	0.96×10^3	0.12×10^3
						av:			
	1					4.94×10^5	3.44×10^5	0.96×10^3	0.12×10^3
	2	5.52×10^6	4.24×10^6	7.80×10^3	4.80×10^3	3.60×10^6	2.64×10^6	1.32×10^3	0.36×10^3
0.3	3	5.80×10^7	6.24×10^7	5.04×10^3	3.96×10^3	6.08×10^7	5.08×10^7	2.52×10^3	1.20×10^3
	4	1.68×10^8	1.56×10^8	1.12×10^4	8.64×10^3	1.16×10^8	9.60×10^7	2.28×10^3	1.20×10^3
	1	1.56×10^3	0.72×10^3	--- ²	--- ²				
	7	1.88×10^6	2.16×10^6	8.76×10^3	1.07×10^4				
	10	2.28×10^7	1.96×10^7	1.03×10^5	3.48×10^4				
	14	3.12×10^8	3.40×10^8	3.96×10^5	4.32×10^5				
0.6	17	7.08×10^8	5.24×10^8	6.40×10^4	8.40×10^4				
	1	0.24×10^3	--- ²	--- ²	--- ²				
	7	1.38×10^3	1.80×10^3	--- ²	--- ²				
	10	1.56×10^6	1.56×10^6	1.80×10^3	1.32×10^3				
	14	1.03×10^7	1.01×10^7	7.52×10^4	9.72×10^4				
	17	1.12×10^7	1.08×10^7	4.96×10^4	4.88×10^4	1.22×10^6	1.28×10^6	1.92×10^3	2.16×10^3
	21	2.96×10^7	2.48×10^7	1.13×10^6	1.23×10^6				
	24	3.00×10^8	2.52×10^8	6.32×10^6	6.64×10^6	1.62×10^6	1.54×10^6	2.04×10^3	0.24×10^3
	31					1.88×10^6	1.90×10^6	3.12×10^3	1.92×10^3
	37					8.52×10^6	8.16×10^6	0.28×10^3	$.24 \times 10^3$

¹Three samples were counted at 0-day to determine the difference in initial contamination existing between samples. No significant differences were found, therefore subsequent samplings were based on one sample only.

²No growth observed.

The results obtained in this second storage test at 42°F were similar to those obtained previously except that the 0.6 megarad samples reached 10^8 by the 24th day as compared with the previous one which had only reached 10^7 by the 32nd day. Spoilage of fillets at 42°F was due primarily to bacterial growth.

Chemical Tests: It has been demonstrated in our previous 33°F and 42°F irradiated yellow perch storage test that the TVA content did not significantly increase until after the product was determined to be spoiled by sensory examination. Since the TVA test has previously been shown to provide more meaningful information on the spoilage pattern of irradiated yellow perch, TVA will be the chemical evaluation employed in subsequent yellow perch storage tests.

The results of the chemical tests (TVA) are presented in Table 4. Volatile acid production in yellow perch fillets irradiated to 0.3 megarad generally increased progressively up to the 15th day of storage. Volatile acid numbers reached high values (40.06) by the 20th day of storage. This appears to correlate with organoleptic results. However, volatile acid production in the fillets irradiated to 0.6 megarad did not show any significant increases throughout the storage test.

Conclusions

The shelf life of yellow perch fillets stored at 42°F is approximately 6 days. Irradiation to 0.3 and 0.6 megarad provides a 3- and 4-fold increase, respectively.

Table 4. — Total volatile acids (TVA) content of yellow perch irradiated at 0.0, 0.3, and 0.6 Mr, and stored at 42°F.

Days in Storage	TVA (VAN)		
	0.0 Mr	0.3 Mr	0.6 Mr
1	5.23	0.27	4.09
3	2.62	0.0	1.34
6	30.70	2.55	4.63
8	51.61	1.27	2.01
10	---	2.95	4.09
13	---	3.62	0.0
15	---	7.85	0.20
20	---	40.06	3.15
24	---	93.67	3.35

Re-Evaluation of Irradiated Yellow Perch
Fillets Stored at 33°F

Objective

To determine the maximum shelf life of yellow perch fillets irradiated to 0.0, 0.3, 0.6 megarad and stored at 33°F.

Materials

Fifty pounds of yellow perch fillets hand-cut from Lake Michigan gillnetted fish (South Haven, Mich., September 1964).

Experimental Procedures

Sensory Evaluation: Preparation, cookery, and presentation of samples remained the same as previously reported except that the frozen reference samples were not irradiated.

Microbiological Examination: The procedures remained the same as previously reported.

Chemical Tests: None conducted.

Results and Discussion

Sensory Evaluation: Table 5 presents the sensory scores for overall acceptability. Non-irradiated samples were unacceptable between the 8th and 13th day of storage. Fillets irradiated 0.3 megarad were unacceptable between the 26th and 33rd day. Little difference was indicated by the sensory panel between fillets irradiated to 0.3 and 0.6 megarad. This may have been due to the use of nonirradiated reference samples.

Table 5. -- Sensory scores for overall acceptability.

Days in Storage	Irradiation dose (megarads)		
	0.0	0.3	0.6
6	7.6	-	-
8	7.1	6.8	6.3
13	2.9	6.4	-
14		-	6.0
19		6.5	-
20		-	6.3
26		5.0	4.8
33		4.5	3.8
42		3.8	4.3

Microbiological Examinations: Table 6 illustrates the variations in total aerobic microbial plate counts, and total yeast and mold plate counts of the unirradiated and nonirradiated yellow perch fillets stored at 33°F.

The initial microbial count (20°C) of the unirradiated samples averaged approximately 8.62×10^5 . By the 5th day of storage the sample had reached a total count of 4.4×10^7 .

Irradiation to 0.3 megarad reduced the total microbial count by 99.9 percent. By the 22nd day of storage it reached a count of 1.32×10^7 .

Irradiation to 0.6 megarad reduced the total microbial count by nearly 100 percent. By the 50th day of storage it reached a count of 1.07×10^7 .

Yeast and mold counts were reduced by both irradiation levels and remained at insignificant numbers throughout subsequent storage. Yeast and mold counts in the 0.6 megarad samples were not obtained until the 40th day of storage. It is interesting to note that the yeasts and molds were more sensitive to irradiation than the ones present in our 33°F storage test conducted during the same season the previous year (September 1963). At that time, we reported yeasts and molds as the principal cause of spoilage in the irradiated samples. Otherwise, the pattern of the gross flora growth curves in the unirradiated and irradiated samples is similar.

Conclusions

The data obtained from this test is generally similar to that obtained from a similar test conducted one year ago (Annual Report to the AEC, 1964) with the exception of the sensory scores for samples irradiated to 0.6 megarad.

Table 6. — Total aerobic microbial plate counts of unirradiated and irradiated yellow perch fillets stored at 33°F.

Storage time (days)	0.0 Mrad				0.3 Mrad				0.6 Mrad			
	Microbial counts		Yeast and molds		Microbial counts		Yeast and molds		Microbial counts		Yeast and molds	
	per gram		per gram		per gram		per gram		per gram		per gram	
	20°C	30°C	20°C	30°C	20°C	30°C	20°C	30°C	20°C	30°C	20°C	30°C
	9×10^5	5.32×10^5	5.16×10^3	5.64×10^3								
	8.86×10^5	5.96×10^5	6.96×10^3	5.64×10^3								
	8.0×10^5	4.28×10^5	4.31×10^3	4.32×10^3								
	av:	av:	av:	av:								
0	8.62×10^5	5.18×10^5	5.48×10^3	5.2×10^3								
1					0.96×10^3	0.36×10^3	0.24×10^3	---	1.56×10^3	---	---	---
4	7.64×10^6	6.32×10^6	2.66×10^4	2.4×10^4								
5	4.4×10^7	4.8×10^7	6.4×10^3	6×10^3								
8					1.33×10^3	0.48×10^3	0.16×10^3	---	1.2×10^3	---	---	---
12					5.16×10^3	---	---	---	0.4×10^3	---	---	---
15					2.87×10^4	3.46×10^4	0.48×10^3	5.52×10^3	---	---	---	---
19					1.56×10^5	1.4×10^5	3.96×10^3	5.88×10^3	0.36×10^3	---	---	---
22					1.08×10^6	9.6×10^5	5.04×10^3	5.76×10^3	4.44×10^3	4.4×10^3	---	---
26					1.32×10^7	1.44×10^7	$>10^3$	$>10^3$	$.48 \times 10^3$	1.25×10^3	---	---
29					2.8×10^6	2.8×10^6	4.4×10^4	3.2×10^4	2.09×10^4	2.16×10^4	$.24 \times 10^3$	0.24×10^3
33					5.88×10^7	6.92×10^7	6.68×10^5	7.48×10^5	1.68×10^3	2.8×10^3	---	---
36					9.2×10^6	9.6×10^6	2.8×10^4	4.4×10^4	3.64×10^4	5.72×10^4	---	---
40					7.6×10^6	6.4×10^6	2.16×10^4	1.84×10^4				
43					1.4×10^7	1.72×10^7	4×10^4		7.16×10^6	7.52×10^6	1.85×10^4	9.36×10^3
47					3.16×10^7	3×10^7	6.44×10^4	7.24×10^4	7.76×10^6	6.28×10^6	3.38×10^4	3.48×10^4
50									2.66×10^6	2.52×10^6	8×10^3	7.2×10^3
54									1.07×10^7	1.12×10^7	2.6×10^4	2.08×10^4
61									1.8×10^7	1.8×10^7	2.72×10^4	2.4×10^4
									$1. \times 10^7$	9.4×10^6	2.88×10^4	2.48×10^4

*No growth observed.

2. Smoked Chubs

Because of the current processing and holding restrictions imposed by the states bordering the Great Lakes as a result of the 1963 botulism outbreaks involving smoked fish, it has become apparent that the application of gamma radiation as a means of extending the refrigerated shelf life in sensory terms only would now have little or no practical application to this product. However, studies of the possible use of the irradiation process as an adjunct to other processing parameters such as time and temperature of heating, salt content, the use of bacteriostatic additives, etc., could result in information of real value to the industry and the consuming public.

At present, the smoked fish industry is experiencing great difficulty in producing a product whose processing conditions conform to state regulations and whose eating quality is acceptable to the consumer. State regulations require that the fish be heated to an internal temperature of 180°F and this temperature be held for 30 minutes. This temperature greatly exceeds that of the traditional process. The use of irradiation might allow for a reduction in the required heating conditions. The possibility that such application might be successful has been indicated by the findings of the Army's Natick Laboratories in that relatively high doses of irradiation can be applied to raw beef products while frozen, resulting in only minor induction of irradiation flavors/odors.

Thus, preliminary studies were initiated at this laboratory to determine if irradiation could be applied to the processing of smoked fish to increase its saleability without detriment to that level of consumer safety attained through the use of current time-temperature processing requirements. These studies were necessarily preliminary in nature and it is anticipated that they will be continued in further depth during the next contract year.

Effect of Brining Time and Brine Strength on Salt Content of Smoked Chubs Produced from Frozen Stocks

Objective

To determine the effect of brining time and brine concentration on the salt content of smoked product from chubs held in storage at 0°F.

Materials

100 pounds of 60° salometer brine, and 80 pounds of medium-size raw frozen chub (gutted, head-on).

Procedure

The initial brine stock was prepared by dissolving 15.6 pounds of salt (NaCl) in 84.4 pounds of tap water (at 60°F). From this, brines of 45°, 30°, and 15° salometer were obtained by

appropriate dilutions. Twenty pounds of brine at each strength were placed in a 33°F refrigerator to attain storage temperature (33°F) prior to brining of fish.

Ten pounds of thawed chubs were placed in the brine to give an initial brine to fish ratio of 2:1. Samples were removed from the brines according to the following schedule.

Sample Removal Schedule			
Brine strength (° salometer)			
<u>15°</u>	<u>30°</u>	<u>45°</u>	<u>60°</u>
1 hour	1 hour	---	---
9 hours	9 hours	9 hours	---
---	24 hours	24 hours	24 hours
---	---	---	42 hours

After brining, one portion of each sample was analyzed for percentage salt, and another portion was smoked to 180°F internal for 30 minutes and then analyzed for salt. Samples for analysis consisted of the skin-on fillet for the brined-only fish, and the dorsal muscle (skin removed) for the smoked fish. Percentage salt was determined according to the Official Methods of Analysis, AOAC, 9th Ed.

Results and Discussion

Table 7 presents the percentage of salt found in raw, brined fish and in finished product from the various brining treatments. Generally, as brining time or brine strength increased, so also did salt content.

Table 7. — Effect of brine strength and brining time of salt content of brined and smoked fish from frozen stocks.

Brining treatment (Salometer/time)	Percentage NaCl	
	Brined-only fish	Smoked fish
15° - 1 hr	0.60	0.82
15° - 8 hr	1.32	1.41
30° - 1 hr	0.93	1.61
30° - 8 hr	1.75	2.06
30° - 24 hr	3.48	3.57
45° - 8 hr	2.18	3.29
45° - 24 hr	4.06	4.29
60° - 24 hr	3.62	4.04
60° - 42 hr	4.43	5.99

Effect of Irradiating Temperature
On Irradiation Flavor Development—Test 1

Objective

To determine the effect upon irradiation flavor development of irradiation of raw fish at either 0° or 33°F at irradiation doses of either 2.0 or 4.5 megarads.

Procedures

All fish were from the same lot, which had been frozen 2 days after capture and held at 0°F until this test was conducted. A portion of the fish was thawed and held at 33°F, during irradiation. The actual dose rates under which the samples were irradiated were 200 Krad/hr for 21.7 hr = 4.34 megarad (high dose samples) and 100 Krad/hr for 21.7 hr = 2.17 megarad (lower dose samples). Another portion was irradiated while in the frozen state. A third portion was thawed and used as a control. After irradiation treatment, all samples were brined in a 25° salometer brine for 18 hours, and then smoked to 150°F internal for 30 minutes. After smoking, the loin muscles were removed, flaked and mixed for presentation to a 4-member sensory panel. Samples were scored for flavor according to a 9-point hedonic scale.

Results

Table 8 presents the results of sensory evaluation of smoked fish samples processed under the 2 irradiating temperatures and to the 2 dose levels. Since a score of 5 or higher must be obtained for acceptable eating quality, it can be seen that, of the treated samples, only those irradiated in the frozen state at these relatively high doses were considered to be acceptable.

Table 8.—Sensory evaluation of smoked chubs irradiated at 0° and 33°F to levels of 2.17 and 4.34 megarads.

Sensory Scores					
Controls	2.17 Mrad		4.34 Mrad		
	33°F	0°F	33°F	0°F	
7	4	8	4	6	
7	5	7	5	5	
9	6	8	6	8	
8	4	3	3	4	
Average	7.8	4.8	4.5	5.8	

Conclusions

Irradiation of raw product in the frozen condition appears to markedly reduce irradiation flavor in smoked chub. High levels of irradiation can be tolerated.

Effect of Irradiating Temperature on Irradiation Flavor Development—Test 2

Objective

To determine the effect on irradiation flavor development in raw chub irradiated at 0°F to dose levels ranging from 0.3 to 1.4 megarads.

Procedure

Four lots of raw, dressed chub were irradiated while frozen at 0°F to 4 dose levels: 0.343, 0.679, 1.028, and 1.461 megarads. After irradiation, the fish were thawed, brined at 35° salometer for 18 hours, and smoked to 180°F for 30 minutes. Nonirradiated controls were thawed, brined, and smoked collectively with treated samples.

After smoking, the loin muscles of the fish from each lot were removed, flaked, and mixed together to provide a reasonably uniform mixture of smoked fish flesh for each treatment. A 5-member sensory panel evaluated the samples for odor, flavor, and overall acceptability. Three samples were presented per session consisting of either 1 control and 2 samples irradiated at the same dose, or 2 controls and 1 treated sample. The panelists were requested to identify the unlike sample and then to evaluate each of the samples using a 9-point hedonic scoring system (a score of 5 or higher indicated acceptance of the product).

Results and Discussion

Table 9 presents the sensory scores obtained on the smoked fish flesh treated in the above manner. The average score for reference samples was 8.2, closely followed by an average score of 7.8 for fish irradiated to 0.343 megarad. Scores for fish irradiated to 0.679-1.461 megarads ranged from an average of 6.4 to 7.0.

Table 9.—Sensory evaluation of smoked chub produced from raw fish irradiated at 0°F to levels ranging from 0.343 to 1.370 megarads.

<u>Panel Member</u>	<u>Reference</u>	<u>0.343 Mrad</u>	<u>0.679 Mrad</u>	<u>1.028 Mrad</u>	<u>1.370 Mrad</u>
1	8	7	7	8	7
2	8	9	7	8	4
3	8	8	7	6	7
4	9	7	4	4	7
5	8	7	7	8	8
Average	8.2	7.8	6.4	7.0	6.6

Thus, although those fish receiving the lowest dose were considered to be most similar to the reference samples in flavor, those fish irradiated at the higher levels were all found to be acceptable.

REFERENCE

Emerson, J. A., N. Kazanas, R. A. Greig, H. L. Seagran, P. Markakis, R. C. Nicholas, B. S. Schweigert, and L. L. Kempe, 1964. Irradiation preservation of fresh water fish and inland fruits and vegetables. Prepared for the Division of Isotopes Development, U.S.A.E.C. Report number COO 1283-12, Isotopes-Industrial Technology, TID 4500 (19th Ed.).

PART B. MICROBIOLOGY OF YELLOW PERCH

1. Isolation and Identification

Studies on the generic identification of bacteria isolated from nonirradiated and irradiated perch samples during the general course of microbial spoilage is reported here. In addition, total yeast and mold counts from these samples are discussed. A detailed knowledge of the processing technique on these flora, is important in establishing irradiation dose level and development of more meaningful criteria to estimate quality loss.

Little work has been reported concerning the microflora of freshwater fishery products of the United States. Bogoslawski (1961) published an abstract on freshwater fish bacteria, however, his findings have not been published in detail. Evelyn and McDermott (1961) reported studies on the microflora of several species of freshwater fish from Ontario—trout species accounted for 97.1 percent of the fish examined; the remainder were "coarse" fish.

The current report presents microbiological data on commercially processed fillets which contained the commensal plus contaminating flora. To our knowledge, this is the first study reported on fresh and spoiling nonirradiated and irradiated freshwater fishery products. It is hoped that the identification of these microflora will complement our previous and current studies on the pattern and significance of gross flora change in relation to quality of untreated and irradiation treated yellow perch fillets.

Methods

Bacteria: Following the enumeration and tabulation of macrocolonies on petri dish culture plates obtained from perch fillets during the general course of microbial spoilage occurring at temperatures of either 33° or 42°F (Emerson et al., 1964); representative colonies from TPY-extract agar plates were selected and keyed out as to generic designation. For this purpose, the isolates were screened into groups employing essentially the morphological and biochemical protocols of Shewan (1960 a) and Masurovsky (1963) to facilitate their grouping as to genus. Our modification of the aforementioned schemes is presented in Figure 1. The isolates were initially screened into groups, then taking one group at a time, their identification was further confirmed by referring to Bergy's Manual (7th Ed. 1957).

FIGURE 1. GENERIC IDENTIFICATION SCHEME FOR BACTERIA ISOLATED FROM PERCH FILLETS

Gram positive organisms have been reported to be inhibited by the dyes in MacConkey's medium (Difco Manual). Because gram positive organisms such as Micrococcus, Corynebacterium and Brevibacterium have been reported to be readily decolorized (Bergey's Manual), all isolates were plated on MacConkey's medium. Inhibition of organisms on this medium plus other morphological and biochemical characteristics helped to identify those gram positive organisms easily decolorized and giving a false gram negative stain.

The data obtained from each isolate was coded and recorded on IBM cards, which through mechanical sorting has further aided these bacterial identifications. These cards constitute a versatile and permanent record in our culture documentation.

Yeasts: The procedure employed for total yeast counts is the same as described in the previous annual report (Emerson et al., 1964).

Results

Bacteria: The total number of organisms of each genus found in the nonirradiated and irradiated fresh and spoiling perch fillets stored at either 33° or 42°F are presented in Table 10. This serves to indicate the gross composition of the bacterial flora found in the products during the entire period of storage. Those organisms found in greatest numbers in the overall sense were Pseudomonas, Micrococcus, Achromobacter, Alcaligenes and Brevibacterium.

Figure 2 presents the average total microbial counts for all tests as determined during the course of storage. Also indicated are those organisms isolated at various times of storage. It can be seen that nonirradiated samples had an initial count of approximately 6×10^5 , whereas, the initial counts of samples irradiated at 0.3 and 0.6 megarad were approximately 4×10^3 and 2×10^3 , respectively. No lag phase was noted in the nonirradiated samples, whereas, a lag period of about 6 and 18 days of storage were observed for those samples irradiated at 0.3 and 0.6 megarad, respectively. Average growth rates for all samples appeared to be the same. It was found that the counts entering the stationary phase were approximately 1×10^8 for the control samples, whereas, the counts of samples irradiated at 0.3 and 0.6 were approximately 1 log number lower.

Organisms found in the nonirradiated perch on initial isolation, in order of decreasing number, were: Pseudomonas, Micrococcus, Brevibacterium, Achromobacter, Alcaligenes, Bacillus, Sarcina, Corynebacterium, Flavobacterium and a few Proteus, Escherichia, and Lactobacillus. A few unidentified isolates resembling mycobacteria (fast growers) and microbacteria were

and will be further investigated. As spoilage progressed, the flora shifted and the pseudomonads became predominant. At the 4th day of storage, there were still a few pigmented bacteria (Micrococcus and Brevibacterium) isolated from the samples, but none were isolated after

FIGURE 2. EFFECT OF IRRADIATION DOSAGE ON GROWTH RATE AND LAG PHASE OF TOTAL MICROBIAL PLATE COUNTS FROM FILLETS STORED AT 33°F, AND ORGANISMS ISOLATED AT DIFFERENT STORAGE TIMES

the 5th day. Micrococci were still present by the 12th day of storage, although the pseudomonads re predominant (Pseudomonas species have been reported to be principally responsible for the spoilage of marine fish).

Irradiation of samples to 0.3 and 0.6 megarad eliminated the most predominant genus, Pseudomonas. A few brevibacteria, micrococci, and yeasts survived these doses, but the predominant organisms isolated throughout the storage period were of the achromobacter-alkaligenes group.

Yeasts: Figure 3 illustrates variations in total yeast plate counts of unirradiated and irradiated perch fillets stored at 33°F. Yeast counts in nonirradiated samples remained at insignificant levels throughout storage. Similar results were observed for irradiated samples, except in one storage test where both the 0.3 and 0.6 megarad samples showed a high level of yeast growth. The flora of these samples exhibited a shorter lag phase than those from Lake Michigan. Sour and yeasty odors were produced from these surviving yeasts. It is interesting to note that the fish used in this particular test came from Lake Erie, whereas, the others came from Lake Michigan. However, the initial yeast contamination was higher in those samples from Lake Michigan as against those from Lake Erie. No explanation for the high yeast outgrowth in irradiated Lake Erie samples is available at this time.

Table 10. — Total number of organisms of each genus isolated from irradiated and nonirradiated perch fillets stored at 33°F and 42°F.

Organisms isolated (Genus)	Storage temperature					
	33°F			42°F		
	Dose (Mr)			Dose (Mr)		
	0.0	0.3	0.6	0.0	0.3	0.6
<u>Pseudomonas</u>	64	0	0	9	4	0
<u>Micrococcus</u>	26	0	0	5	0	0
<u>Achr-Alcalig</u>	20	39	10	3	13	5
<u>Brevibacterium</u>	21	1	1	1	0	0
<u>Sarcina</u>	6	0	0	2	0	0
<u>Corynebacterium</u>	4	0	0	2	0	0
<u>Bacillus</u>	8	0	0	2	1	0
<u>Escherichia</u>	1	0	0	0	0	0
<u>Proteus</u>	3	0	0	0	0	0
<u>Flavobacterium</u>	3	0	0	0	0	0
<u>Lactobacillus</u>	1	0	0	0	0	0
<u>Yeast</u>	0	7	2	1	0	0

Proteolytic Activity of Bacterial Isolates

Organisms from spoiling fish, which grow at low temperatures and exhibit proteolytic activity, have been assumed to be primarily responsible for spoilage (Shewan, Hobbs, and Hodgkiss, 1960b).

FIGURE 3. TOTAL YEAST PLATE COUNTS FROM IRRADIATED AND NONIRRADIATED PERCH FILLETS OF THREE STORAGE TESTS (Sept. 1963, Feb. 1964, Sept. 1964) AT 33°F

In order to evaluate the proteolytic activity of organisms isolated from yellow perch, a fish substrate which would be readily available and could be easily sterilized was developed. To preserve the native state of the heat labile constituents, cold sterilization was necessary. Although filtration-sterilization could accomplish cold sterilization, the various difficulties that would be involved removed this method from consideration. Thus, a raw perch-extract was sterilized through the use of irradiation and then combined with sterile agar to provide the final medium. Preliminary results showed that this medium could be used as a suitable experimental substrate for the study of bacterial proteolytic activity during fish muscle spoilage.

During the general course of microbial spoilage, irradiated and nonirradiated bacteria which were isolated and identified were also screened for proteolytic activity on sterile, raw perch-extract agar medium and confirmed using the conventional caseinolytic activity test on a skim milk agar.

Materials

Perch-extract agar medium: Perch was frozen immediately after catch, then dressed and skinned. The flesh was ground and pressed. For the latter operation, a Carver laboratory press, Model B was utilized. The pressed juice was passed through a double thickness of Whatman No. 40 filter paper on a Büchner funnel. The pressed juice was then sterilized by Co^{60} gamma radiation to a dose of 3.0 megarads while being held at 33°F. Incubation of this extract at 20° and 30°C for as long as a month showed no microbial growth. Fish-extract agar plates were prepared by adding 100 ml of the sterile fish-extract to 1 liter of sterile 1.5 percent agar, holding both ingredients at 42°C prior to mixing.

30 percent skim milk medium: Commercially prepared sterile evaporated skimmed milk concentrated 2-fold was employed. A 30 percent skim milk agar medium was prepared by adding 15 cc of the skimmed milk to 85 cc of sterile nutrient agar (0.3 percent bacto-beef-extract, 0.5 percent bacto-peptone, and 1.5 percent agar) held at 42°C and poured into petri dishes.

Procedure

Isolates were streaked on the perch-extract agar, and on skim milk agar and incubated at 20°C for 24 to 48 hours. After this period, plates were reincubated at 30°C, showing this temperature to be more suitable for proteolytic activity of some organisms. Optimum growth temperature for all isolates was found to be between 20° and 30°C. Caseinase or fish muscle proteases could be adaptive enzymes, being elaborated only as needed, thus stimulation of these extracellular enzymes sometimes happens under repeated transfer to fresh substrate. Therefore, those isolates (after primary isolation from TPY medium) showing negative activity in

her milk or fish-extract agar were retransferred to fresh milk or fish-extract agar, respectively, which could finally result in the utilization of the substrate. Organisms showed proteolytic activity on these media by a clearing zone in the medium surrounding the isolate.

Results

Table 11 shows the number of proteolytic bacteria isolated from nonirradiated and irradiated perch. Proteolytic activity on skim milk and perch-extract agar was demonstrated by some of the pseudomonads, brevibacteria, micrococci, sarcina, corynebacteria, and yeasts. Most of the organisms showing proteolytic activity were those isolated from nonirradiated perch—particularly the pseudomonads which became predominant while spoilage progressed. Of the 38 pseudomonads tested, 33 showed proteolytic activity. Irradiation to 0.3 and 0.6 megarad eliminated essentially all proteolytic bacteria. The only irradiated organism isolated from perch stored at 33°F showing proteolytic activity on both media was an unidentified pink yeast isolated in TPY immediately after irradiation and during the first 8 days of sample storage. The yeasts isolated on malt agar were not tested for proteolytic activity on either media.

The irradiated fillets stored at 42°F showed few pseudomonads, bacillis, and brevibacteria surviving irradiation, but they were no longer evident by the 3rd day of fillet storage:

All isolates plated on the irradiation-sterilized perch extract medium exhibited good growth in 24 hours. Of those organisms showing proteolytic activity, most exhibited this characteristic on both media. However, a few showed proteolytic activity either on the perch extract or on the skim milk agar. Of a total of 233 isolates tested, 54 were found to be positive for proteolytic activity. Of these, 42 were positive on both media, an additional 8 were positive only on the skim milk, and 4 others were positive only on the perch extract.

Table 11.—Proteolytic activity of isolates from irradiated and nonirradiated yellow perch fillets stored at 33° and 42°F.

Storage temp. (°°)	Dose (Mrad)	Number isolated ¹	Total	Pigm.	Non-Pigm.	Pseudomonas	Brevibacterium	Micrococcus	Sarcina	Bacillus	Corynebacterium	Yeast	Unidentified	Lost
33	0.0	79	32	20	12	18	6	4	1	0	1	0	1	1
	0.3	53	6	6	0	0	0	0	0	0	0	6	0	0
	0.6	39	2	2	0	0	0	0	0	0	0	2	0	0
42	0.0	30	8	5	3	1	0	3	2	0	1	1	0	0
	0.3	23	4	1	3	3	0	0	0	1	0	0	0	0
	0.6	9	2	1	1	0	1	0	0	1	0	0	0	0
totals		233	54	35	19	22	7	7	3	2	2	9	1	1

¹Yeast initially isolated on malt agar are not included in this table.

3. Conservation of Bacterial Isolates

Several excellent methods are available for preserving microorganisms (Martin, 1964) and with many organisms there is a choice of at least two proven techniques. Lyophilization is the method most commonly used for long-term conservation. The advantages of lyophilization, especially in the maintenance of large numbers of cultures, are considerable (Haynes, 1955 and 1963). Several factors have been shown to influence the survival of microorganisms in the lyophilized state (Fry, 1954; Greaves, 1960).

In the current investigations, bacterial isolates from irradiated and nonirradiated yellow perch fillets were lyophilized and stored following their identification.

Materials

The lyophilizing menstruum employed was "mist dessicans" (Fry and Greaves, 1951; Fry, 1954) prepared with 3 parts serum and 1 part peptone broth plus 10 percent glucose. The serum was employed to form a "solid dry cake." Glucose was added as a buffer to hold the residual moisture at about 1 percent (Muggleton, 1963). Ten percent Difco-peptone and 10 percent glucose were mixed together after being sterilized and the mixture employed for suspending the cultures to be lyophilized. Bovine serum was prepared from whole blood obtained from a local abattoir and sterilized by Seitz filtration.

Procedure

Mature cultures at the maximum stationary phase were employed. When cultures were pigmented, harvesting was done after pigment was fully developed. To the mature culture, 2 cc of peptone-sugar mixture were added, the culture loosened with the pipette, and the organisms suspended. To a sterile ampule containing 0.8 cc of sterile bovine serum, 0.25 cc of the peptone-sugar cell mixture was added. The ampules were loosely plugged with cotton and freezing carried out in two stages. The suspension was first frozen in powdered dry ice, rotating the ampule to form a shell of the cell material—cooling to about -20°C . The ampule was then transferred to an ice-acetone mixture to rapidly lower the temperature to approximately -50°C (Muggleton, 1963). Cells were then lyophilized utilizing a Virtis "Bio-Dryer Unitrap" apparatus. Lyophilization of the bacterial preparations required about 3-4 hours, after which the ampules were sealed under vacuum. Lyophilized cultures were then stored at $3-5^{\circ}\text{C}$.

Conclusions

The pseudomonas, which were found to be greater in number in the nonirradiated product and the predominant toward spoilage of the fillets, were essentially eliminated by irradiation to 0.3 and 0.6 megarad. Irradiation to these levels eliminated the proteolytic bacteria, leaving a residual flora composed of the achromobacter-alcaligenes group.

Preliminary results showed that perch-extract agar could be used as a suitable experimental substrate for the study of microbial proteolytic activity during fish muscle spoilage.

REFERENCES

- Bergey's Manual of Determinative Bacteriology, 1957. 7th Ed. Williams and Wilkins Co., Baltimore, Maryland.
- Bogoslowski, A. S., 1961. Fisheries Research. IV. Fresh-water fish bacteria. Canadian Fisherman (Natl. Business publication, Ltd., Gardenvale (Que., Canada), 48(7):34(a).
- Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures. 1953. 9th Ed., Difco Laboratories, Inc., Detroit, Michigan.
- Duggan, D. E., A. W. Anderson and P. R. Elliker. 1963. Appl. Microbio., 11:398-403.
- Emerson, J. A., N. Kazanas, R. A. Greig, H. L. Seagran, P. Markakis, R. C. Nicholas, B. S. Schweigert and L. L. Kempe. 1964. Irradiation preservation of fresh water fish and inland fruits and vegetables. Prepared for the Division of Isotopes Development, U.S.A.E.C. Report number COO 1283-12, Isotopes-Industrial Technology, TID4500(19th Ed.).
- Evelyn, T. P. T., and L. A. McDermott. 1961. Bacterial studies of fresh water fish. I. Isolation of aerobic bacteria from several species of Ontario fish. Can. J. of Microbio (National Research Council, Ottawa, Canada), 7:375-82.
- Fry, R. M. 1954. The Preservation of Bacteria in Biological Appl. of Freeze and Drying, Ed. R. J. C. Harris, N. Y., Academic Press.
- Fry, R. M., and R. I. N. Greaves, 1951. J. Hyg., 49:220-46 (1951).
- Greaves, R. I. N. 1960. Recent Research in Freezing and Drying, Parkes, A. S., and Smith, A. U., Eds., Blackwell, Oxford.
- Haynes, W. C., L. J. Wickerham and C. W. Hesseltine. 1955. Appl. Microbiol., 3:361-68.
- Haynes, W. C. 1963. Culture Collections: Perspectives and Problems, Martin, S. M., Ed., University of Toronto Press, Toronto.
- Lerke, P., R. Adams, and L. Farber. 1963. Bacteriology of spoilage of fish muscle, Appl. Microbiol., 11:458-462.
- tin, S. M. 1964. Conservation of microorganisms. Ann. Rev. Microbiol. 18:1-16.
- Masurovsky, E. B., J. S. Voss and S. A. Goldblith. 1963. Changes in the microflora of haddock fillets and shucked soft shelled clams after irradiation with Co^{60} gamma rays and storage at 0°C and 6°C . Appl. Microbiol. 11(3):229-238.

Muggleton, P. W. 1963. Progr. Ind. Microbiol., 4:191-214.

Shewan, J. M., G. Hobbs, and W. Hodgkiss. 1960 a. A determinative scheme for the identification of certain genera of gram-negative bacteria, with special reference to the pseudomonada-ceal. J. Appl. Bact., 23(3):379-390.

Shewan, J. M., G. Hobbs and W. Hodgkiss. 1960 b. The Pseudomonas and Achromabacter groups of bacteria in the spoilage of marine whitefish. J. Appl. Bact., 23:463-468.

PART C. THE EFFECT OF GAMMA RADIATION ON ANTIMETABOLITES CONTAINED IN FISH

Introduction

Thiaminase, an antithiamine factor indigenous to certain species of fish, is undesirable in food and feed products made from fish containing this antimetabolite. Due to the current interest and possible use of irradiation in processing industrial fishery products, it is important to know the effect of gamma radiation on this enzyme.

The thiaminase-active fish that are currently being used for animal foods are usually processed by cooking at high temperatures to destroy the antithiamine factor. Cooking alone, however, is often detrimental to the acceptability and nutritive quality of the product. The simultaneous use of gamma radiation and mild heating, or even high levels of radiation alone, could likely serve as alternate approaches to inactivate the enzyme without thoroughly cooking the product.

Initial tests at this laboratory showed that the thiaminase of alewife viscera could be destroyed by gamma radiation. In view of these results, investigations were started under this contract to study more thoroughly the effect of radiation on this enzyme. The principal objective of this study was to determine the radiosensitivity of thiaminase in situ in various species of fish under controlled conditions of pH and temperature.

Methodology

Radiation processing of industrial fishery products would most likely be done on whole fish and whole fish wastes. Thus, the irradiation was carried out in such a manner as to simulate industrial conditions. Following irradiation, samples were assayed for residual thiaminase activity by standardized procedures.

Preparation of samples: Fresh whole fish were ground once through a 1/4-in. plate, mixed, and then ground once through a 1/8-in. plate. The ground material was then packed and sealed in metal TDT cans (about 20 grams capacity), and held at 0°C until irradiated. These cans

proved superior to plastic bottles that were used initially. Control of sample size and homogeneity, which is an important factor in these comparative studies, was thus improved.

Irradiation of samples: The canned fish samples (contained in an insulated cylinder) were placed in the center well of the cobalt-60 source. Temperature control during irradiation was accomplished by packing either dry ice or crushed ice above and below the samples in the insulated cylinder. Room temperature irradiation was carried out at ambient temperature of the source. Irradiation was done at the Phoenix Memorial Laboratory located on the campus of The University of Michigan.

Analysis of samples: Thiaminase activity of all the samples was determined by the assay procedure of Gnaedinger (1965). This procedure consists of first blending a known amount (5-10 grams) of thiaminase-active fish in a buffered KCl solution. The homogenate is then mixed with a known amount of thiamine hydrochloride. Enzyme activity is then determined by following, fluorometrically, the rate of thiamine destruction. Thiaminase activity curves are shown in Figures 4 and 5, relating effect of dosage level to residual activity at irradiation temperatures of 33° and 90°F, respectively.

Investigations

In Table 12 are given the variables that were studied under this project (species, pH, and temperature). Various species were used in order to determine whether differences existed between species in the radiosensitivities of their respective thiaminases. Since slight differences in natural pH were noted between species, one test was conducted to determine the effect of this variable on samples of whole alewife that were adjusted to pH 8.1 and 4.6 with NaOH and HCl, respectively. The combined effect of irradiation and temperature was also studied.

Summary of Results

Thiaminase activity of whole fish was not reduced when samples of whole alewife, carp, and shad were packed in dry ice and irradiated to 6 megarads; however, there are indications that alewife at pH 8.1, whole smelt, and spottail subjected to the same conditions are slightly radiosensitive.

At 33°F, up to 50 percent reduction occurred in some samples when irradiation was carried up to 4 megarads.

Still greater reductions were obtained when the samples were irradiated at room temperature and up to 4 megarads.

Changes in pH, above and below the normal pH, did not increase the radiosensitivity of thiaminase. In fact, there appeared to be a slight protective effect.

FIGURE 4. EFFECT OF GAMMA RADIATION ON THIAMINASE ACTIVITY OF WHOLE ALEWIFE, pH 6.90, 33°F

FIGURE 5. EFFECT OF GAMMA RADIATION ON THIAMINASE ACTIVITY OF WHOLE ALEWIFE, pH 6.90, 85°-95°F

Results indicate that the thiaminases of different species may not be equally sensitive to gamma radiation.

Table 12. — The effect of gamma radiation on fish thiaminase.

Species	pH	Dosage (megarads)	Observed reduction in thiaminase activity ²		
			<u>-0° F³</u>	<u>33° F</u>	<u>85°-95° F</u>
Alewife	6.90 ¹	6	0	-	-
Carp	6.75 ¹	6	0	-	-
Shad	6.65 ¹	6	0	-	-
Smelt	6.85 ¹	6	Slight reduction	-	-
Shiner	6.70 ¹	6	"	-	-
Alewife	4.5	4	0	20	-
Alewife	8.1	4	Slight reduction	26	-
Alewife	6.90	4	-	56	80
Shad	6.65	4	-	13	43
Smelt	6.85	4	-	52	95

¹Natural pH.

²Expressed as a percentage of the activity of unirradiated control samples.

³Samples irradiated in dry ice.

Discussion

The principal conclusions drawn from this study are: (1) radiation has no apparent effect on thiaminase when irradiated in situ in the frozen state, and (2) the combined use of temperature and radiation shows the most promise for possible adaptation to commercial processing of industrial fish. The proposed next year's study includes a detailed examination of the latter relationship, which will involve temperatures up to 180° F.

Two other interesting observations were made, both of which could warrant additional study. First, there appeared to be differences in the thiaminases of different species, judging from their different radiosensitivities. The general composition of the fish or the concentration of activators or inhibitors could, however, account for this variation. Secondly, the apparent protective effect of pH levels above and below the natural pH is at present unexplained. Further study in this area, using purified enzyme systems, will be necessary.

REFERENCE

edinger, R. H. Thiaminase activity of fish: An improved assay method. *Fishery Industrial Research* (In press).

PART D. PUBLICATIONS AND PRESENTATIONS

Effect of gamma irradiation on the microflora of fresh water fish. I. Microbial load, lag period and rate of growth on yellow perch (Perca flavescens). Nuria Kazanas, J. A. Emerson, H. L. Seagran, and L. L. Kempe. (In preparation.) To be submitted to Food Technology.

Irradiation preservation of fresh water fish. I. Extension of refrigerated storage life of fresh yellow perch fillets. J. A. Emerson, Nuria Kazanas, R. A. Greig, H. L. Seagran, and L. L. Kempe. (In preparation.) To be submitted to Food Technology.

Effect of gamma irradiation on the microflora of yellow perch fillets. Nuria Kazanas. Presented January 9, 1965, at the Winter Meeting of the Michigan Branch of the American Society for Microbiologists, Wayne State University, Detroit; at the Eighth Conference on Great Lakes Research, Ann Arbor, March 29-30, 1965; and at the Sixteenth Annual Meeting of the Pacific Fisheries Technologists, Sidney, B. C., Canada, March 21-24, 1965.

Microbiological studies on irradiated yellow perch (Perca flavescens) fillets — gross population changes and isolation of aerobic bacteria. Nuria Kazanas. Presented at the 65th Annual Meeting of the American Society for Microbiologists, Atlantic City, April 25-29, 1965.

Irradiation preservation of fresh water fish — Irradiation methodology, product parameters, and sensory approach. J. A. Emerson. Presented at the Eighth Conference on Great Lakes Research, Ann Arbor, March 29-30, 1965.

Section 2

IRRADIATION PRESERVATION OF FRUITS AND VEGETABLES

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SUMMARY

- Gamma irradiation (200 krad) of strawberries, var. Robinson, will delay by 2-3 days the rapid decay caused by transportation without refrigeration and subsequent storage at 44°F.
2. Overwrapping with polyethylene film may add to the quality protection of Robinson strawberries afforded by 200 krad gamma irradiation.
 3. This year no quality benefits were apparent on sweet cherries exposed to 150 krad gamma irradiation.
 4. The dehydration of prunes is facilitated by gamma irradiation (200-400 krad).
 5. Dry beans are softened by exposure to radiation up to 1.6 Mrad.
 6. It is possible to reduce the yeast plus mold counts of apple juice to 0.2% of the original count by irradiation (250 krad) and storing the juice under anoxic condition.
 7. An effort to extract more sugar from sugar beets by irradiation failed. However, some arresting of the browning of the shredded beets was observed.
 8. The sprouting of Arenac and Ontario potatoes was inhibited by 7.5 krad of gamma irradiation.
 9. The resistance to radiation of Aspergillus flavus spores, suspended in water, is rather low ($D \approx 45$ krad); the pH shows no effect, and glucose increases it slightly. Dry spores are more radioresistant than spores in ag. suspension.
 10. Low dose irradiation (200-600 krad) releases calcium from plant tissues.

REPORT

During the second year of research under the AEC contract a number of experiments on the effect of irradiation on fruits and vegetables were carried out. The following is a rather condensed description of these experiments.

STRAWBERRIES

Two experiments on strawberry irradiation were carried out during the 1964 season. The objective of the first experiment was to see whether irradiation could allay the effect of transport on strawberries. The second experiment was aimed at studying overwrapping in combination with irradiation.

1. Shipment experiment. Robinson strawberries grown near Detroit were placed in 168 wooden pint boxes and transported to the nearby irradiation facility in cardboard shippers containing 12 pint boxes each. Overripe, under-ripe damaged fruit was excluded. For irradiation, 84 pint boxes were arranged in two 3-layer rings around the cobalt-60 source. The inner ring received 50 krad/hr and the outer ring 25 krad/hr. For uniform dose distribution, each box was turned by 180° around a vertical axis at half-dose time. The total dose was 200 krad. After

irradiation, the boxes were placed in the shippers, transported to the lab. in East Lansing, 66 miles away, and stored at 44°F. The control strawberries were handled similarly. The day following irradiation, the firmness of the irradiated and control fruit was tested by means of the Lee-Kramer shear press. For each testing 100 grams of fruit was placed in the cell. Figure 1 is a typical automatically recorded texture curve. The sharp peak is interpreted as indicating the shear-pressure, while the smoother part of the curve probably corresponds to the compression of the fruit. Seven such tests were performed for the irradiated berries and seven for the controls.

Two days after irradiation, all shippers were loaded on a station wagon and transported 234 miles to simulate commercial shipping of Michigan-grown strawberries. The fruit reached a temperature of about 80°F during transportation and it was placed in the 44°F room subsequently.

At intervals of time indicated in Table 1 the texture of irradiated and control fruit was tested exactly as it was done one day after irradiation. The results are summarized in Table 1. The irradiated fruit was generally softer, although the difference was not statistically significant on the 1st and 9th day.

Table 1.—Shear testing of control and irradiated (200 krad) strawberries at various times after irradiation. The numbers are peak Press readings and indicate maximum shear pressure.

Days After Irradiation	Control	Irradiated
1	52.8	43.3
3	62.3	47.7**
6	62.4	47.7**
8	65.1	53.9*
9	59.0	51.0

*5% probability level.

**1% probability level.

The effect of irradiation and shipping on the taste of the fruit was also tested with the participation of 15 tasters. Both the triangular and the pair comparison tests were used five times during the 15-day post-irradiation storage. No significant difference could be detected between irradiated and control strawberries.

The berries exhibiting lesions (mold growth or mushy spots) were also counted in samples of seven pints of fruit periodically and the results are presented in Figure 2.

This experiment indicates that 200 krad gamma irradiation of Robinson strawberries will delay the damage caused by transportation without refrigeration and subsequent storage at rather high temperature (44°F) by 2 to 3 days.

FIGURE 1. LEE-KRAMER SHEAR PRESS TRACING OF TWO 100-GRAM SAMPLES OF STRAWBERRIES

FIGURE 2. EFFECT OF GAMMA RAYS AND SHIPPING ON LESION APPEARANCE OF STRAWBERRIES

2. Overwrap experiment. Robinson strawberries grown near Detroit were exposed to 200 krad of gamma rays in pint boxes, at The University of Michigan irradiator. There were groups of 156 boxes each. Two groups were irradiated, one of them with a 1.5 mil polyethylene wrap around each box, and the other without overwrap. Similarly, there were two groups of nonirradiated berries, one with and the other without an overwrap around each box.

After irradiation, the irradiated and control fruit was transported without refrigeration to Michigan State University, 66 miles away, and placed in a cooler at 42°-45°F. During storage at this temperature the following observations and tests were made.

Color. No color difference between irradiated and control berries was noticeable during the period of observation. This confirms previous results in which instrumental evaluation of color was used. There was more gloss on the strawberries in overwrapped boxes, both irradiated and control, than on those without the cover. Before unwrapping, however, the condensation on the inside of the film rendered the covered boxes somewhat less attractive.

Lesions. At periods of time indicated in Table 2, thirteen boxes were removed from cold storage, the berries in them were weighed and those with one or more visible lesions were removed and weighed in order to determine percent spoilage in weight. The spoilage judge, a person trained in food microbiology, did not know the identity of the fruit group she was working on. The results are presented in Table 2 and indicate that under the conditions of this experiment spoilage can be delayed 2 to 5 days by 200 krad gamma irradiation. Among the two non-irradiated fruit groups, that without overwrap suffered the greatest losses, while the irradiated and overwrapped fruit displayed the least spoilage of all four groups.

Table 2. — Effect of gamma irradiation (200 krad) and subsequent storage at 44°F on the spoilage of strawberries. Numbers indicate sound fruit on % weight basis (sample size, 13 pints).

Overwrapped			Non-overwrapped		
Days After Irradiation	Control	Irrad.	Days After Irradiation	Control	Irrad.
1	97.2	99.8	1	96.6	95.3
3	86.0	96.4	3	94.4	94.1
5	83.3	93.6	5	78.8	94.0
7	66.0	18.7	7	60.8	70.4
8	55.2	81.7	8	44.6	73.8
9	--	--	9	30.8	62.4
10	--	--	10	25.4	66.4
11	44.1	81.1	11	19.4	48.6
12	--	--	12	--	--
13	--	--	13	--	--
14	13.6	47.1	14	12.2	24.9
15	11.1	35.6	15	7.1	14.3
17	4.2	33.5	17	--	--
19	--	--	19	--	--
21	0	15.4	21	--	--

Texture. The firmness of the fruit was measured by means of the Lee-Kramer shear press imes indicated in Table 3. For each testing 100 grams of sound fruit were placed in the sample cell and 12 or 13 such tests were performed on each group every day of testing. The means of the maximum press readings were treated statistically and are presented in Table 3. Irradiation resulted in softening of the fruit and in spite of a remarkable recovery of firmness (about 50% in 13 days) the irradiated fruit remained softer than the control fruit throughout the storage period, since the control fruit also firmed up during storage.

Table 3. Effect of gamma irradiation and subsequent storage at 44°F on the texture of strawberries. Numbers indicate shear press peak readings (average of 12-13 tests).

Overwrapped				Non-overwrapped			
Days After Irradiation	Cont.	Irrad.	Diff.	Days After Irradiation	Cont.	Irrad.	Diff.
1	45.7	36.4	9.3**	1	48.2	35.7	12.5**
3	53.2	43.0	10.2**	3	51.8	37.8	14.0**
5	55.5	45.5	10.0**	5	59.8	44.2	15.6**
7	57.8	49.6	8.2**	7	59.1	46.6	12.5**
9	59.3	45.5	13.8**	9	60.5	47.6	12.9**
11	57.6	48.0	9.6**	11	60.2	52.6	7.6*
13	--	50.2	--	13	--	53.9	--
15	--	--	--	15	--	--	--
19	--	--	--	19	--	--	--

**1% probability level.

*5% probability level.

Taste. For taste comparisons 15 judges were used in a triangular test on the 1st, 3rd, and 7th day after irradiation. The berries were sliced and mixed with sugar at the ratio one sugar, ten fruit. No significant difference was found between unwrapped irradiated and unwrapped control berries. Wrapped control strawberries scored significantly lower than wrapped irradiated berries, after one day of post-irradiation storage. Apparently an off-flavor was developed in the wrapped control fruit.

This experiment indicates that overwrapping with thin polyethylene film may add to the quality protection of Robinson strawberries afforded by 200 krad gamma irradiation.

SWEET CHERRIES

Sweet cherries of the Schmidt variety, grown in western Michigan, were hand-picked, placed in wooden pint boxes and transported by automobile to the gamma irradiation on site at Ann Arbor, 3 miles away, without delay. There were four groups of fruit, each group composed of 156 pint boxes. Two groups were exposed to 150 krad irradiation, one of them with a 1.5 mil polyethylene wrap around each box, the other without overwrap. Similarly, there were two groups of nonirradiated cherries, one with the other without over wrap around each box.

After irradiation, all of the fruit was shipped to E. Lansing and stored at 38°-40°F. During storage the following observations and tests were made.

Color. No color difference between the four groups of cherries was noticeable.

Lesions. At periods of time indicated in Table 4, 13 boxes were removed from each group and the percent of spoiled cherries in them was estimated, on a weight basis. The results indicate that irradiation decreased the spoilage rate very slightly and only in the case of the over-wrapped fruit.

Table 4. Effect of gamma irradiation (150 krad) and subsequent storage at 39°F on the spoilage of sweet cherries. Numbers indicate sound fruit on % weight basis (sample size, 13 pints).

Overwrapped			Non-overwrapped		
Days After Irradiation	Control	Irrad.	Days After Irradiation	Control	Irrad.
1	100.0	100.0	1	100.0	100.0
3	93.5	95.7	4	92.6	95.4
6	93.8	89.9	6	87.7	91.2
8	88.6	90.1	8	87.7	91.2
10	87.6	83.2	11	81.5	85.9
13	80.5	84.7	14	67.2	73.9
16	80.7	77.7	18	60.4	67.0
20	73.6	70.6	21	56.4	56.4
24	59.6	56.5	25	47.4	47.0
31	33.2	32.4	28	47.9	51.2
38	31.0	26.8	32	33.9	43.6
			35	30.5	34.6

Texture. The texture of the cherries was measured by means of the Lee-Kramer shear press, using a technique similar to that for strawberries. A statistically significant softening due to irradiation was found, corresponding to approximately 20% decrease of the peak press reading during the first week and 10% during the 3rd and 4th weeks.

Taste. Triangular taste testing in which 15 judges participated showed no significant effect of the irradiation on the taste of the non-overwrapped cherries. The control wrapped cherries, however, were preferred over the irradiated wrapped cherries.

No overall advantage appears to be derived by irradiating (150 krad) sweet cherries, according to this experiment. It is possible, however, that the unusually dry season resulted in low microbial contamination of the fruit and the anti-microbial effect of irradiation was not demonstrable.

PRUNES

Michigan-grown Stanley prunes were exposed to 0, 50, 100, 200 and 400 krad of gamma rays, dipped in 200°F water for 30 seconds and dried in a Proctor dehydrator, at 155°F air temperature.

FIGURE 3. EFFECT OF GAMMA RAYS ON THE DRYING RATE OF STANLEY PRUNES

FIGURE 4. EFFECT OF GAMMA IRRADIA ON THE TEXTURE OF NAVY BEANS

The rate of dehydration increased as the irradiation dose increased. Under the conditions of this experiment the time to reach 25% moisture content for prunes exposed to 200 krad of gamma rays is approximately 3/4 the time for the controls (Figure 3). Although some red pigment leached out of the irradiated (200 and 400 krad) fruit during blanching, the anthocyanin content of the dry control prunes was approximately 2/3 that of the irradiated fruit. The skin of the prunes which were exposed to 200 and 400 krad broke during blanching. No flavor difference was detected between irradiated and control dry samples by a panel of 15 tasters. When the blanching was omitted no acceleration of the drying rate by irradiation was observed and no increased anthocyanin retention.

Irradiation may prove complimentary to dehydration and freeze-drying of foods.

DRY NAVY BEANS

1. Dry navy beans were exposed to several doses of gamma rays in the range of 0 to 1.6 Megarad, subsequently soaked in 4 times their weight of distilled water for 24 hours and tested by the Allo-Kramer shear press. A sharp reduction in the firmness of the soaked beans was observed as the radiation dose increased (Figure 4). The soluble solids of the soak water increased from 1.5% for the control beans to 7.0% for the beans exposed to 1.6 Megarad (Figure 5). Upon rehydration for 24 hours, the weight gain of the nonirradiated beans was 90%, that of beans exposed to 450 krad 101%, and the moisture content 54% and 56% respectively.

2. Dry navy beans were exposed to 10, 100, 400, 600, 800, and 1,000 krad of gamma rays, soaked for 16 hours and cooked in distilled water. After 0, 0.5, 1.0, and 2.0 hours of cooking the weight of the beans was determined. Table 5 summarizes the results.

Table 5.—Effect of gamma irradiation on the water uptakes of dry beans after soaking (16 hrs) and cooking. Figures indicate % of weight increase.

Dose (krad)	0	200	400	600	800	1,000
Soaking only	92.0	96.0	100.0	102.1	84.4	104.1
Soaking + 1/2 hr cooking	97.3	124.0	125.1	118.8	116.7	114.5
Soaking + 1 hr cooking	127.1	139.6	140.7	134.4	125.1	126.1
Soaking + 2 hrs cooking	144.8	144.6	164.5	141.7	135.5	133.3

It is apparent that exposure to 400 krad of gamma rays results in maximum water uptake, in general.

3. Navy beans from the market contained 10.4% moisture. One sample was dried to practically 0% moisture by vacuum, and other samples were hydrated to 19.5%, 29.3% and 43.2% moisture by soaking 2, 15 and 60 minutes, respectively. All 5 samples were exposed to 400 krad of gamma rays, and after soaking for 16 hours the water uptake and the hardness (Allo-Kramer press) of the beans was determined. The results are summarized in Table 6.

FIGURE 5. EFFECT OF GAMMA IRRADIATION ON SOLUBLE SOLIDS OF NAVY BEANS

Table 6.— Effect of gamma rays (400 krad) and moisture content of beans on their water uptake and hardness (Allo-Kramer press peak reading) after soaking.

% Moisture	Control		400 krad	
	% wt. Gain	Hardness	% wt. Gain	Hardness
0.0	90.5	74.3	91.3	43.7
10.4	91.6	79.3	106.3	36.3
19.5	91.6	79.6	100.0	41.7
29.3	94.8	76.5	103.1	42.8
43.2	93.7	74.8	97.9	51.7

The normal moisture was associated with the highest water uptake and softness after irradiation.

4. Dried beans, var. Great Northern, were exposed to 0, 300, and 600 krad of gamma irradiation and subsequently soaked for 16 hours in either equal weight of water or four times as much water. After soaking, the excess water was removed and beans were steam-blanching for three minutes. Eight ounces of these beans were placed in no. 300 cans, covered with pork and bean sauce and the cans were exhausted, sealed, and retorted for 15, 30, 45, 60 and 75 minutes at 240°F. After retorting, the cans were cooled with water, stored at room temperature and opened 2 days later. The following observations were made on the canned beans.

1. The irradiated samples were the softest when short periods of retorting were used, but this tendency reversed as retort time increased.
2. Equal weights of soak water and beans yielded the softest beans.
3. Drained weight increased with radiation dose at brief retorting, but the reverse held true at long retort times.
4. Equal weights of beans and soak water yielded the highest drained weights.
5. There was not much difference in color development between all treatments.
6. The viscosity of the sauce in the beans soaked with an equal weight of water increased with radiation dose after 15 min. of retorting. Longer retorting reversed the trend with the sauce from the unirradiated control being the most viscous at 75 min. retorting.
7. In the case of beans soaked with an excess of water the difference in viscosity of the sauce were less pronounced.

APPLE JUICE

Juice from Michigan-grown apples, var. Northern Spy, was exposed to gamma rays in 4-ounce bottles and subjected to the following tests.

Yeast and mold count. Table 7 summarizes the results of plating one day after irradiation.

Table 7. — Effect of irradiation on the yeast and mold count of apple juice.

<u>Dose</u>	<u>Count/ml</u>	<u>% Reduction</u>
0	1400	0
100	437	68.8
200	157	88.8
300	26	98.1
400	0.5	99.9
500	0	100.0

Color. Visual observation indicated a progressive lightening in the color of the juice with increasing radiation dose. Upon storage at 50°F the irradiated juice darkened more rapidly than the control and after one month of storage there was little difference in color between the samples.

Flavor. Preliminary tasting tests on the effect of the lower doses of irradiation on the flavor of the juice were inconclusive. When larger samples of juice were exposed to 300 krad of gamma rays and compared with heat-pasteurized and non-heated juice, after one week storage of all 3 samples at 50°F, the panel found the irradiated juice inferior to the other two types of juice.

Viscosity. Table 8 summarizes the juice viscosity measurements, as determined by the Ostwald viscosimeter.

Table 8. — Effect of gamma irradiation on the viscosity of apple juice.

<u>Dose</u>	<u>Rel. Viscosity</u>
0	2.91
100	2.82
200	2.65
300	2.34
400	2.20
500	2.18

2. Local apples of the Northern Spy and McIntosh varieties were mixed and pressed in the laboratory. The juice was depectinized, clarified and packed in sterile glass bottles. The air of a number of filled bottles was removed by means of a high vacuum pump and nitrogen, free of oxygen, replaced the air in the bottles. The sealed bottles were exposed to 0, 150, 200 and 250 krad of gamma rays.

Five days after irradiation, the juice was tasted by a 15-member untrained panel and no significant difference in taste was detected between levels of irradiation, or between air and nitrogen atmospheres.

After five days of storage at 50°F, samples of the juice were plated for mold plus yeast enumeration. The results are summarized in Table 9.

Table 9. — Mold plus yeast count (per ml.) of apple juice irradiated under air or nitrogen and stored at 50°F for five days.

Control		150 krad		200 krad		250 krad	
Air	N ₂	Air	N ₂	Air	N ₂	Air	N ₂
9650	-	4850	410	820	52	88	21

These results indicate that irradiation and storage of apple juice under nitrogen results in great reduction of the mold plus yeast count.

3. Non-clarified apple juice was exposed to 0, 100, 200, 300, 400 and 500 krad of gamma rays and subsequently stored at 50°F. The periodic measurements of the viscosity of the juice are summarized in Table 10.

Table 10. — Effect of gamma irradiation and storage at 50°F on the viscosity of non-clarified apple juice. The figures are minutes of flow through an Ostwald viscosimeter.

Sample	0 wk.	1 wk.	2 wks.	4 wks.
0 krad	3.429	2.697	2.725	1.700
100 krad	3.200	2.968	2.412	2.046
200 krad	2.951	2.697	2.397	2.046
300 krad	2.615	2.480	2.249	2.161
400 krad	2.514	2.452	2.219	2.103
500 krad	2.343	2.976	2.160	2.233

These results indicate that gamma rays decrease the viscosity of apple juice immediately after irradiation. Upon storage, however, the decrease in viscosity of nonirradiated juice is more rapid than that of irradiated juice; this is probably due to pectolytic activity of microorganisms since a clarified, nonirradiated sample showed very little change of viscosity during similar storage.

4. Clarified apple juice was irradiated and stored as in (3). Part of the juice was packed under air and part under nitrogen. Periodically, the color of the juice was measured by diluting it with 3 volumes of water and determining the absorbancy of the dilution at 440 m in an Evelyn colorimeter. The results are summarized in Table 11.

Table 11. — Effect of gamma irradiation and storage at 50°F on the color of air and nitrogen packed, clarified apple juice. The values are A₄₄₀.

Sample	0 wk.		1 wk.	2 wks.	4 wks.	
	Air	N ₂			Air	N ₂
0 krad	.240	.230	.231	.189	.192	.182
100 krad	.181	.142	.174	.201	.161	.147
200 krad	.128	.107	.114	.123	.54	.137
300 krad	.096	.093	.111	.087	.125	.125
400 krad	.091	.086	.101	.087	.129	.124
500 krad	.090	.088	.101	.097	.119	.173

Both the air-packed and nitrogen-packed juice is bleached by irradiation, but upon storage the difference in color decreases as the irradiated juice darkens and the control juice becomes lighter in color.

5. Non-clarified juice was irradiated and stored as in (3). Periodically 1 volume of juice was mixed with 3 volumes of 95% ethanol, the mixture was filtered and the color of the filtrate was measured at 440 m μ in the Evelyn colorimeter. Table 12 summarizes the results.

Table 12. — Effect of gamma irradiation and storage at 50°F on the color of apple juice filtrate. (Non-clarified juice was used which was depectinated by ethanol just before color measurement.) The values are A₄₄₀.

Sample	0 wk.	1 wk.	2 wks.	4 wks.
0 krad	.335	.248	.233	.096
100 krad	.277	.222	.204	.114
200 krad	.229	.206	.164	.102
300 krad	.177	.150	.123	.087
400 krad	.143	.183	.118	.095
500 krad	.133	.195	.141	.079

Generally, irradiation and storage, independently resulted in bleaching of the ethanolic filtrate. The juice, however, did not lighten to the same extent visual observation indicated that the juice was much darker than the filtrate and the sediment obtained by mixing with ethanol was very dark.

SUGAR BEETS

Sugar beets were exposed to gamma rays in the dose range of 0 to 400 krad. They were then shredded and extracted with boiling water. No significant difference was found in the total soluble solids extracted, nor in the optical rotation of the extracts.

It was observed, however, that the shredded beets exposed to 50 and 100 krad irradiation darkened very little in comparison to the nonirradiated ones or those irradiated with higher doses.

POTATOES

1. Potatoes of the Arenac and Ontario varieties were exposed to 0, 2.5, 5.0, 7.5, and 10 krad of gamma rays and subsequently stored at 70°F, 45% RH for conditioning prior to making potato chips. The potatoes were evaluated periodically for weight loss, sprout inhibition, physical appearance, reducing sugar content and chipping quality.

a) Wt. loss. The results on weight loss appear in Tables 13 and 14.

b) Sprouting. Two days after irradiation sprouting could be observed in the 0, 2.5, and 5.0 krad treatments. Potatoes exposed to higher levels of irradiation displayed swelling of the buds

but this was not developed to sprouting. The weight of the sprouts after 55 days of storage at 55°F, 45% RH is given in Table 15.

Table 13. — Weight loss of irradiated Arenac potatoes, expressed as % of original weight.

Days of Storage	Control	2.5K	5.0K	7.5K	10.0K
1	99.6	99.4	99.6	99.7	100.0
2	99.1	98.8	98.8	99.9	99.4
3	98.8	98.4	98.4	98.5	98.9
6	97.6	97.0	97.1	97.4	97.6
9	96.6	95.8	95.9	96.6	96.6
13	95.1	94.0	94.4	95.2	95.2
16	93.9	92.6	93.4	94.5	94.2
21	92.1	90.5	91.9	93.1	92.8
27	88.4	96.3	88.7	90.8	90.3
34	85.3	82.7	86.2	89.0	88.2
41	82.0	78.7	83.3	87.0	86.2
55	75.2	71.1	78.2	83.7	82.5

Table 14. — Weight loss of irradiated Ontario potatoes, expressed as % of original weight.

Days of Storage	Control	2.5K	5.0K	7.5K	10.0K
1	99.7	99.7	99.5	99.7	99.6
2	99.2	99.1	98.4	98.7	98.7
3	98.6	98.8	98.3	98.1	98.2
6	97.3	97.2	96.7	96.5	96.7
9	95.8	95.8	95.3	95.0	95.5
13	94.3	93.5	93.7	93.4	93.9
16	93.3	92.6	92.4	92.3	92.5
21	91.5	90.7	90.9	90.4	91.2
27	88.3	87.2	87.7	87.8	88.8
38	85.7	84.1	84.7	85.5	86.5
41	83.0	81.0	81.7	83.2	84.5
55	77.0	74.8	75.8	79.5	80.9

It appears that no significant differences in weight loss between control and irradiated occurred under the conditions of this experiment.

Table 15. — Sprout weight in % of total potato weight after 55 days of storage.

Variety	Control	2.5 krad	5.0 krad	7.5 krad	10.0 krad
Arenac	7.7	6.4	0.8	0.0	0.0
Ontario	5.4	4.9	2.7	0.0	0.0

2. Potato chips were made from the conditioned potatoes which had been treated as described previously. The following standard conditions were used. One half pound of sliced potatoes were fried for 3 minutes at 375°F. After draining the potatoes were graded according to the "Proposed Color Reference Standard" presented at the Production and Technical Division Meeting, National Potato Chip Institute, 1954. The reducing sugar content of the potatoes was also determined. Tables 16 and 17 summarize the results.

Table 16. — Reducing sugar content of Arenac potatoes and color of chips at various irradiation doses and post-irradiation storage periods (70°F).

Post-Irradiation Storage, Days	Control		2.5 krad		5.0 krad		7.5 krad		10.0 krad	
	%	Chip	%	Chip	%	Chip	%	Chip	%	Chip
	Sugar	Color	Sugar	Color	Sugar	Color	Sugar	Color	Sugar	Color
2	1.51	710	1.61	710	1.75	710	1.60	710	1.55	710
8	1.28	9.5	1.27	710	1.46	710	1.37	710	1.48	710
15	.97	9.0	.71	7.5	.85	8.5	.78	9.5	1.03	710
23	.67	8.5	.87	8.5	.77	8.5	.79	9.0	.83	710
30	.65	8.0	.66	7.0	.59	9.0	.68	8.0	.90	9
37	.54	6.0	.49	7.0	.98	9.0	.40	7.0	.39	8
44	.54	8.0	.27	6.0	.34	7.0	.27	6.0	.47	8
58	.59	5.0	.55	6.0	.61	7.0	.79	9.0	.80	8

Table 17. — Reducing sugar content of Ontario potatoes and chip color at various irradiation doses and post-irradiation storage periods (70°F).

Post-Irradiation Storage, Days	Control		2.5 krad		5.0 krad		7.5 krad		10.0 krad	
	%	Chip	%	Chip	%	Chip	%	Chip	%	Chip
	Sugar	Color	Sugar	Color	Sugar	Color	Sugar	Color	Sugar	Color
2	.52	8.5	.59	9.0	.58	8.0	.56	8.0	.60	8.5
8	.53	7.0	.36	7.0	.47	8.5	.63	8.0	.53	8.5
15	.30	6.5	.33	6.5	.32	6.5	.45	8.0	.50	8.0
23	.35	6.0	.37	6.5	.30	6.5	.35	5.5	.38	6.0
30	.39	6.0	.22	5.0	.30	7.0	.34	7.0	.22	5.0
37	.37	6.0	.30	6.0	.22	5.0	.24	5.0	.33	7.0
44	.18	5.0	.20	6.0	.21	7.0	.18	4.0	.24	6.0

It appears that irradiation, especially at the 10 krad dose, delays the conditioning of potatoes in terms of potato chip color. There is no clear relationship between reducing sugar content and chip color in the irradiated samples.

ASPERGILLUS FLAVUS

1. Two-week old spores of Aspergillus flavus were suspended in demineralized water and exposed to 0, 50, 100, 150, 200, 250 and 300 krad of gamma rays. The percent survival of spores, as measured by plate counting, is presented in Figure 6. No data are given for doses higher than 150 krad because the counts were too few to be significant. An approximate D value of 45 krad can be calculated indicating that A. flavus spores are rather sensitive to irradiation.

2. Two-week old spores of A. flavus were suspended in 0.075 M citrate buffers, pH 3, 4, 5, 6 and 7 and exposed to gamma rays in the dose range of 0 to 200 krad. The survival results presented in Figure 7. It is apparent that pH has no appreciable effect on the sensitivity of the spores to irradiation.

3. Two-week old spores of A. flavus were suspended in demineralized water containing 0.01% Triton X-100 and irradiated with 1-Mev electrons, in Petri dishes. The lids of the dishes

FIGURE 6. EFFECT OF GAMMA RAYS ON
ASPERGILLUS FLAVUS IRRADIATED IN
 DEMINERALIZED WATER

FIGURE 7. EFFECT OF GAMMA RAYS ON ASPERGILLUS FLAVUS SPORES, IRRADIATED IN .075M CITRATE BUFFER OF VARIOUS pH

were removed just before irradiation and replaced immediately after it; control dishes indicated that the contamination ensured using this technique was negligible. The results of the irradiation on the viability of the spores, as determined by plate counting, are summarized in Figure 8. In this figure, the results obtained in a parallel experiment in which gamma irradiation was used are also indicated. No significant difference in the effect of the two types of irradiation on the viability of the A. flavus spores is apparent.

4. Spores of A. flavus were suspended in glucose solutions to give a final concentration of 0, 10, 20, 30 and 40% w/v of the sugar. The spore suspensions were exposed to 1 Mev electrons at the dose levels of 0, 50, 100 and 200 krad.

The results are illustrated in Figure 9, and indicate that dextrose in solution increases the resistance of A. flavus spores to irradiation.

5. Two-week old spores of A. flavus were suspended in demineralized water containing 0.01% Triton X-100. One ml. of this suspension was transferred to each of 14 sterile Petri dishes which were then dried under 27" of vacuum for 4 hours at 25°C and placed in a desiccator overnight. The following day these dishes, along with another 14 dishes which contained 1 ml. of spore suspension plus 3 ml. of water, were exposed to 0, 50, 100, 150, 200, 250 and 300 krad of 1 Mev electrons. The irradiation was performed as described in (3). The results of the irradiation on the ability of the spores to form colonies are summarized in Figure 10. It is apparent that the dried spores of A. flavus are considerably more resistant than the spores which were in water suspension.

6. Two-week old spores of A. flavus were suspended in 0, 10, 20, 30 and 40% solutions of glucose, w/v. The concentration of the spores was 6×10^6 per ml., approximately. The suspensions were exposed to gamma rays from Co-60 in the dose range of 0-200 krad. The effect of the irradiation on the ability of the spores to form colonies is illustrated in Figure 11. It appears that glucose in solution decreases the radiosensitivity of the spores only slightly. The protective effect of glucose against damage by 1 Mev electrons was somewhat greater.

From the same harvest of A. flavus spores, water suspension and dried spores were exposed to gamma rays in the range of 0-300 krad. The results are summarized in Figure 12. The dry spores were more resistant to radiation damage than those suspended in water. It appears, however, that the increase in radioresistance was not so high as that observed when 1 Mev electrons were used. Since the dry spores used in the gamma and electron irradiations were not portions of the same spore preparation the difference might not be entirely due to the nature of irradiation.

FIGURE 8. EFFECT OF IRRADIATION ON ASPERGILLUS FLAVUS SPORES IRRADIATED IN DEMINERALIZED WATER

FIGURE 9. EFFECT OF 1 MEV ELECTRONS ON THE SPORES OF ASPERGILLUS FLAVUS IRRADIATED IN DEXTROSE SOLUTIONS

FIGURE 10. EFFECT OF 1 MEV ELECTRON IRRADIATION ON DRY AND WET SPORES OF ASPERGILLUS FLAVUS

FIGURE 11. EFFECT OF GAMMA IRRADIATION ON THE SPORES OF *ASPERGILLUS FLAVUS* IRRADIATED IN VARIOUS DEXTROSE SOLUTIONS

FIGURE 12. EFFECT OF GAMMA IRRADIATION ON DRY AND WET SPORES OF ASPERGILLUS FLAVUS

RADIATION-INDUCED CALCIUM RELEASE FROM PLANT TISSUES

Softening of fruits and vegetables upon exposure to ionizing radiations has been repeatedly reported. This softening has been associated with changes in the pectic substances of the irradiated tissues. The association has been clear, however, only for high (over 500 kilorad) doses of irradiation. The effect of lower doses of irradiation on the texture of plant tissues, although strong, has not been interpreted in well defined chemical changes. Since lower doses (200 to 300 kilorad) are likely to be used in the radiation-pasteurization of fruits and vegetables, it is important to understand the mechanism of the radiation-induced softening. Such understanding may facilitate the search for means to avoid the softening in the cases in which it is undesirable, or enhance it when desirable.

The premise on which the following experiments were undertaken has been that calcium significantly contributes to the firmness of fruits and vegetables, specifically through the Ca-pectin association. Should calcium be released by irradiation the texture of the plant tissue would be expected to weaken.

In a preliminary experiment, dried tomato leaves containing ^{45}Ca were pulverized, extracted with 70% ethanol and the marc was divided into two equal-weight portions. One portion was suspended in water and exposed to 595 kilorad of gamma rays from Cobalt-60, while the other portion was also suspended in water but not irradiated. After the solid phase of each suspension was separated by filtration, an aliquot of the filtrate from the irradiated marc was found to display 4,146 counts per min. (cpm) of radioactivity, but an equal aliquot of the control filtrate showed only 633 cpm.

In a second experiment, a semi-ripe strawberry fruit was severed from the plant and the stem of the berry was immersed in a solution of $^{45}\text{CaCl}_2$ (approx. 5 μc). Two days later the berry was crushed in boiling 95% ethanol, disintegrated in a Waring blender, filtered and the marc was washed with 70% ethanol until very little radioactivity could be detected in the washings. The marc was then divided into two equal portions and each portion was suspended in 5 ml. of water. One of the suspensions was exposed to 200 kilorad of gamma irradiation in a glass ampoule and the other was kept as a control; they were then filtered and the marc was again washed with 200 ml. of 70% ethanol. Filtrate and washings were mixed and the solvent was evaporated; the dry residue was dissolved in 4 ml. of 1N HCl and 0.5 ml. of the solution was transferred into a planchet, dried and measured for radioactivity by a gas-flow, thin window detector, connected with an automatic sample changer, scaler and printing timer. The radioactivity of the irradiated sample was 1,646 cpm, that of the control was 593 cpm.

In a third experiment, ^{45}Ca -labelled strawberry marc was prepared by the same method as in experiment No. 2. The suspension of the marc was irradiated in an uncovered Petri dish

with 200 kilorad of 1 Mev electrons, rather than gamma rays. The radioactivity of the non-irradiated control was 350 cpm, that of the irradiated sample 908 cpm.

The higher radioactivity of the extracts of the irradiated plant tissues can not be due to induced radioactivity, because no such induction is expected from the radiation energy levels used here. It must be due to radiation-induced release of bound calcium from the tissues. In order to show that pectin-bound calcium can be released by ionizing radiations the following experiment was conducted.

Calcium-45 pectate was prepared by precipitation of pectic acid from an aqueous solution with $^{45}\text{CaCl}_2$. The precipitate was washed with water until the radioactivity of the washings reached a practically constant value. The marc was dried in a desiccator and portions equal to 60 mg. each were suspended in 3 ml. of water. One suspension was exposed to 200 kilorad of gamma rays, a second one to 200 kilorad of 1 Mev electrons and a third suspension was not irradiated. The control and irradiated suspensions were filtered, the solid phase was washed with 100 ml. of water and the filtrate plus washings were prepared for radioactivity measurements by the procedure used in the strawberry experiments. The relative radioactivities were 73 cpm for the control, 114 cpm for the sample exposed to gamma rays and 143 cpm for the sample exposed to 1 Mev electrons.

These results indicate that calcium can be released by irradiation of plant tissues and this release may be associated with the radiation-induced softening of fruits and vegetables.

Section 3
IRRADIATION OF FOODS

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FOR

Division of Isotopes Development
United States Atomic Energy Commission

CONTRACT NUMBER

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DISCUSSION

When the present research project was initiated, two cobalt-60, gamma radiation sources were available. These were described in the previous annual report for this project (1). It soon became apparent that the quantities of fish, fruits and vegetables to be irradiated for research proposed on this project were more than could be properly accommodated. The cobalt-60 was old and the irradiation zones were not designed to maintain food at refrigeration temperature during irradiation. With these facts in mind, the AEC authorized a supplement to the present contract that permitted both of these deficiencies to be corrected.

New cobalt-60 was installed in both of the UM sources, and the irradiation room at the Phoenix Radiation Facility (PRF) was air conditioned. Similar refrigeration and air conditioning equipment is presently being installed in the Phoenix Memorial Laboratory Source (PML). The strengthened PRF and PML sources now contain 4700 and 10,900 curies of cobalt-60 respectively. They provide maximum gamma ray dose rates of approximately 0.2 megarad per hour at the PRF and 1.0 megarad per hour at the PML. Lower dose rates than the maxima can be conveniently obtained at both sources by conducting irradiations outside of and at calibrated distances from the sources.

The air conditioning equipment is designed to maintain the air spaces in the irradiation chambers at 37°F. This should permit most products to be irradiated without icing; if ice is used, it should last for the length of most irradiation periods required by the research problems under way or contemplated for this contract.

It is expected that the air conditioning work now in progress at the PML will be finished early this summer. This should complete the installation and provide excellent irradiation equipment for most of the research work in progress.

REFERENCE

- (1) Irradiation Preservation of Fresh-Water Fish and Inland Fruits and Vegetables. Report No. COO 1283-12; Isotopes-Industrial Technology, UC23; TID 4500 (19th Ed.).

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AUG 2 1965

MASTER

IRRADIATION PRESERVATION OF FRESH - WATER FISH AND INLAND FRUITS AND VEGETABLES

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SUMMARY OF RESULTS

Results obtained during this second year of contract effort continue to indicate that irradiation pasteurization is able to significantly extend the refrigerated storage life of fresh-water fish as well as inland fruits and vegetables. The following quotations, taken from this report, illustrate these statements and other pertinent findings:

Yellow Perch:

Tests were conducted to confirm previous estimations of the extension of refrigerated storage life of yellow perch fillets. For fillets held at 33°F, the extension of storage life at 0.3 and 0.6 megarad was approximately 4- and 5-fold, respectively. Holding fillets at 42°F decreased the above maximum storage times by approximately 50 percent. It may be concluded that yellow perch fillets are amenable to the irradiation pasteurization process. Significant extension of storage life at 33°F can be attained at a dosage level of 0.3 megarad with only little detriment to initial quality.

Strawberries:

Gamma irradiation (200 krad) of strawberries, var. Robinson, will delay by 2-3 days the rapid decay caused by transportation without refrigeration and subsequent storage at 44°F.

Prunes:

The dehydration of prunes is facilitated by gamma irradiation (200-400 krad).

Potatoes:

The sprouting of Arenac and Ontario potatoes was inhibited by 7.5 krad of gamma radiation.

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FOREWORD

For this effort, The University of Michigan provides irradiation services and general administration of the contracts through the Institute of Science and Technology. This is an arrangement of convenience. Each subcontractor, namely the Bureau of Commerical Fisheries and Michigan State University, is independently responsible for the scientific aspects of its respective research effort.

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INTRODUCTION

Irradiation pasteurization continues to demonstrate potential usefulness for preservation of fresh-water fish and inland fruits and vegetables. This is being demonstrated with fish caught in the Great Lakes and with fruits and vegetables grown in the State of Michigan. The fish are being evaluated by scientists at the U. S. Bureau of Commercial Fisheries Laboratories (BCF) in Ann Arbor, while the fruits and vegetables are being studied in the Department of Food Science at Michigan State University (MSU). Most of the irradiations are carried out in the cobalt-60 gamma ray sources belonging to The University of Michigan (UM), although a resonance transformer at MSU is also used occasionally for this purpose.

The degree of success attained by various treatments for preserving foods is evaluated by organoleptic, chemical and microbiological tests. Through the present contract effort, both equipment and personnel have been added which materially enhance the capacity of these research groups to test and evaluate irradiated foods. Furthermore, the cobalt-60 sources at the UM have been enlarged and equipped for irradiation at refrigeration temperatures.

Since the work is being carried out by three different groups, this report accordingly consists of three sections, each section having been prepared by the group concerned.

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Section 1

IRRADIATION PRESERVATION OF FRESH-WATER FISH

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Subcontract No. 1 under Prime Contract AT(11-1)-1283

(University of Michigan, Ann Arbor)

May 1965

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SUMMARY

The current contract, Subcontract No. 1 under Prime Contract No. AT(11-1)-1283, was initiated April 15, 1963, between the Bureau of Commercial Fisheries Technological Laboratory, Ann Arbor, Michigan, and the Institute of Science and Technology, The University of Michigan. The objective of the study is to investigate the application of cobalt-60 gamma irradiation at sub-sterilization doses to the extension of the refrigerated shelf life of the two most commercially significant Great Lakes fishery products, fresh yellow perch fillets and smoked chub, and the potential usefulness of gamma radiation for controlling the activity of enzymatic antimetabolites of fish and fishery products.

Tests were conducted to confirm previous estimations of the extension of refrigerated storage life of yellow perch fillets. For fillets held at 33°F, the extension of storage life at 0.3 and 0.6 megarad was approximately 4- and 5-fold, respectively. Holding fillets at 42°F decreased the above maximum storage times by approximately 50 percent. It may be concluded that yellow perch fillets are amenable to the irradiation pasteurization process. Significant extension of storage life at 33°F can be attained at a dosage level of 0.3 megarad with only little detriment to initial quality.

Microbiological studies were conducted concurrently on the above products. The initial microbial load on commercially hand-filleted yellow perch fillets, which usually was between 10^5 and 10^6 , was reduced approximately 99 percent by 0.3 megarad and nearly 100 percent by 0.6 megarad of gamma irradiation at 33°F. The microbial lag period was increased by radiation. The degree of increase was a function of dosage; longer lag periods were obtained at the higher radiation dose. Increases in dosage did not decrease the growth rate of the microbial population.

Studies on the generic identification of bacteria were conducted and the methodology is discussed. Organisms found in the nonirradiated perch on initial isolation in order of decreasing number were: Pseudomonads, Micrococcus, Brevibacterium, Achromobacter, Alcaligenes, Bacillus, Sarcina, Corynebacterium, Flavobacterium, and a few Proteus, Escherichia, and Lactobacillus. As spoilage progressed, the pseudomonads became increasingly predominant.

Irradiation of samples to 0.3 and 0.6 megarad eliminated the pseudomonads. The residual flora during storage was primarily comprised of the achromobacter-alcaligenes group.

A suitable experimental substrate for the study of microbial proteolytic activity during fish spoilage is discussed. Proteolytic activity was demonstrated by some of the pseudomonads, brevibacteria, micrococci, sarcina, corynebacteria, and yeasts. Irradiation eliminated essentially all proteolytic bacteria.

Procedures used for the conservation of bacteria through the utilization of lyophilization are presented.

Studies were conducted to determine the effect of irradiating temperature on the irradiation flavor development in smoked chub. It was found that the irradiation of raw chub at 0°F, as against 33°F, significantly reduced irradiation flavors imparted to the product.

Whole fish samples were irradiated at various dosage levels under various conditions of temperature and pH, to determine the effect of these variables on the radiosensitivity of thiaminase in situ. Radiation had no apparent effect on the enzyme when the samples were irradiated in the frozen state. At temperatures above freezing, thiaminase was destroyed by irradiation. Thiaminase appeared to be least stable at the natural pH of the fish tissue. There appeared to be slight differences in the radiosensitivity of the thiaminase of different species.

INTRODUCTION

This annual report represents the second year of effort in accordance with the terms of Subcontract Number 1 under Prime Contract Number AT(11-1)-1283, entitled "Irradiation Preservation of Freshwater Fish" initiated April 15, 1964, between the Bureau of Commercial Fisheries Technological Laboratory, Ann Arbor, Michigan and the Institute of Science and Technology, The University of Michigan, Ann Arbor. The overall objectives of the study are to investigate the application of cobalt-60 gamma irradiation at substerilization doses to the extension of the refrigerated shelf life of the two most commercially significant Great Lakes fishery products; fresh fillets of yellow perch (Perca flavescens) and smoked chub (Coregonus hoyi), and to determine the potential usefulness of gamma irradiation for controlling the activity of enzymatic antimetabolites of fish and fishery products. The conclusions made in this report are tentative as all phases of this study are continuing.

PART A. SHELF LIFE STUDIES

1. Yellow Perch

Re-Evaluation of Irradiated Yellow Perch Fillets Stored at 42°F

Objective

To confirm the results of a previous and similar test to estimate the shelf life of yellow perch fillets irradiated to 0.0, 0.3, and 0.6 megarad and stored at 42°F.

Materials

Thirty-five pounds of commercially hand-cut yellow perch fillets produced from Lake Michigan gillnetted fish (South Haven, Mich., April 1964).

Procedures

All procedures remained essentially the same as previously reported (Emerson et al., 1964) for the comparable test with the following exceptions: (1) sensory evaluation of odor was conducted on raw samples as well as cooked samples, (2) chemical evaluation was limited to TVA (total volatile acids) determinations.

Results and Discussion

Sensory Evaluation: Tables 1 and 2 present the sensory scores from odor evaluation of raw and cooked fillets, respectively. Little difference was noted between the results of the two methods of testing. Unirradiated fillets were unacceptable by the 6th day of storage. Fillets irradiated to 0.3 and 0.6 megarad were found to be unacceptable after 15 and 20 days, respectively.

Several problems in evaluation were encountered during the course of the test. Primarily, the panel members indicated difficulty in determining strict differences between reference and treatment samples without allowing hedonic considerations to influence their decisions. Also, the changes in odor that develop during storage in unirradiated samples are dissimilar from those which develop in irradiated samples. Thus, a change in odor of a certain magnitude in an unirradiated sample may give a grossly different indication of the degree of spoilage which has occurred in that sample than would an identical magnitude of change in an irradiated sample. In many instances the panel scored both the unirradiated sample and an irradiated sample the same, yet the panel considered (hedonically) the former spoiled and the latter still acceptable.

Table 1. —Sensory evaluation¹ of irradiated yellow perch fillets stored at 42°F—Raw fillets.

Days in Storage	Odor Scores (Raw)		
	0.0 Mr	0.3 Mr	0.6 Mr
2	10	6	3.7
6	3.7	8.3	7.0
8	--	7.7	6.0
10	--	5.3	5.0
15	--	6.8	6.0
20	--	5.0	6.4
24	--	--	3.3

¹Based on 9-point hedonic scale, where: 9=like extremely, 1=dislike extremely. A score of 5 or higher indicates acceptance of product.

Table 2. —Sensory evaluation of irradiated yellow perch fillets stored at 42°F—Cooked fillets.

Days in Storage	Odor Scores (Cooked)		
	0.0 Mr	0.3 Mr	0.6 Mr
2	8.6	6.7	8.3
6	3.3	7.3	7.7
8	--	7.0	6.7
10	--	8.0	5.0
15	--	5.0	6.7
20	--	4.7	4.4
24	--	--	5.0

I was fortunate to have an occasion to discuss this general problem with Dr. T. H. Simpson, Torrey Research Station, during his recent stay in this country. He provided a number of useful suggestions in regards to increasing the meaningfulness of our sensory evaluations. As a result, future evaluations of yellow perch fillets will be based entirely on the 9-point hedonic scale as the method of scoring, and the triangle method of sample presentation. After initial establishment of degree of preference between unirradiated versus irradiated samples, each individual triangulation shall be limited to one test variable—time in refrigerated storage. That is, unirradiated samples will be compared only to unirradiated references, and irradiated samples only to irradiated references.

Microbiological Examination: Table 3 illustrates the variations in total aerobic microbial plate counts, total yeasts and molds plate counts of the air-packed unirradiated and irradiated yellow perch fillets stored at 42°F. A preliminary report of the results obtained from the vacuum-packed samples (0.0 Mrad and 0.6 Mrad) is also included.

The initial microbial count (20°C) of the unirradiated air-packed samples was an average of approximately 1.86×10^5 and the psychrophilic count (3°C) was approximately 5.95×10^4 . By the 4th day of storage, the sample had reached a total count of approximately 1.68×10^8 at both incubation temperatures (20°C and 3°C). Similar values were obtained in the vacuum-packed samples. The yeasts and molds total counts increased only 10-fold during the 4 days of storage and only an insignificant increase was observed in the vacuum-packed samples.

Irradiation to 0.3 megarad reduced the total microbial count by approximately 99.2 percent. Initial unirradiated values in the air-packed samples were exceeded by the 5th day of storage and reached a total of 3.12×10^8 by the 14th day. Psychrophilic (3°C) total counts followed the same growth pattern. Unfortunately, vacuum-packed samples at this radiation level were omitted due to the limited sample capacity in the irradiator.

Irradiation to the 0.6 megarad level reduced the total microbial count by nearly 100 percent and the count progressively increased to reach a count of 3×10^8 by the 24th day of storage in the air-packed samples. By the 37th day of storage the vacuum-packed had only reached a count of 8.52×10^6 and at that time the test was terminated due to depletion of irradiated vacuum-packed samples.

Yeast and mold counts of the samples were reduced by both irradiation levels. At 0.3 megarad the counts remained at insignificant numbers and at 0.6 megarad the counts were reduced nearly 100 percent during the first 7 days of storage reaching then a count of 6.32×10^6 by the 24th day of storage. The counts in the vacuum-packed samples did not increase as expected throughout subsequent storage.

Dose (Mr)	Storage time (days)	Air-Packed				Vacuum-Packed			
		Microbial counts		Yeasts and molds		Microbial counts		Yeasts and molds	
		per gram		per gram		per gram		per gram	
		20°C	3°C	20°C	3°C	20°C	3°C	20°C	3°C
0.0		1.68×10^5	5.60×10^4	0.96×10^3	0.12×10^3				
		1.48×10^5	5.92×10^4	1.56×10^3	0.12×10^3				
		2.44×10^5	6.32×10^4	0.96×10^3	0.36×10^3				
		av:							
	0 ¹	1.86×10^5	5.95×10^4	1.16×10^3	0.20×10^3				
						5.80×10^5	2.60×10^5	0.96×10^3	0.12×10^3
						4.08×10^5	4.28×10^5	0.96×10^3	0.12×10^3
						av:			
	1					4.94×10^5	3.44×10^5	0.96×10^3	0.12×10^3
	2	5.52×10^6	4.24×10^6	7.80×10^3	4.80×10^3	3.60×10^6	2.64×10^6	1.32×10^3	0.36×10^3
	3	5.80×10^7	6.24×10^7	5.04×10^3	3.96×10^3	6.08×10^7	5.08×10^7	2.52×10^3	1.20×10^3
	4	1.68×10^8	1.56×10^8	1.12×10^4	8.64×10^3	1.16×10^8	9.60×10^7	2.28×10^3	1.20×10^3
0.3	1	1.56×10^3	0.72×10^3	--- ²	--- ²				
	7	1.88×10^6	2.16×10^6	8.76×10^3	1.07×10^4				
	10	2.28×10^7	1.96×10^7	1.03×10^5	3.48×10^4				
	14	3.12×10^8	3.40×10^8	3.96×10^5	4.32×10^5				
	17	7.08×10^8	5.24×10^8	6.40×10^4	8.40×10^4				
0.6	1	0.24×10^3	--- ²	--- ²	--- ²				
	7	1.38×10^3	1.80×10^3	--- ²	--- ²				
	10	1.56×10^6	1.56×10^6	1.80×10^3	1.32×10^3				
	14	1.03×10^7	1.01×10^7	7.52×10^4	9.72×10^4				
	17	1.12×10^7	1.08×10^7	4.96×10^4	4.88×10^4	1.22×10^6	1.28×10^6	1.92×10^3	2.16×10^3
	21	2.96×10^7	2.48×10^7	1.13×10^6	1.23×10^6				
	24	3.00×10^8	2.52×10^8	6.32×10^6	6.64×10^6	1.62×10^6	1.54×10^6	2.04×10^3	0.24×10^3
	31					1.88×10^6	1.90×10^6	3.12×10^3	1.92×10^3
	37					8.52×10^6	8.16×10^6	0.28×10^3	$.24 \times 10^3$

¹Three samples were counted at 0-day to determine the difference in initial contamination existing between samples. No significant differences were found, therefore subsequent samplings were based on one sample only.

²No growth observed.

The results obtained in this second storage test at 42°F were similar to those obtained previously except that the 0.6 megarad samples reached 10^8 by the 24th day as compared with the previous one which had only reached 10^7 by the 32nd day. Spoilage of fillets at 42°F was due primarily to bacterial growth.

Chemical Tests: It has been demonstrated in our previous 33°F and 42°F irradiated yellow perch storage test that the TVA content did not significantly increase until after the product was determined to be spoiled by sensory examination. Since the TVA test has previously been shown to provide more meaningful information on the spoilage pattern of irradiated yellow perch, TVA will be the chemical evaluation employed in subsequent yellow perch storage tests.

The results of the chemical tests (TVA) are presented in Table 4. Volatile acid production in yellow perch fillets irradiated to 0.3 megarad generally increased progressively up to the 15th day of storage. Volatile acid numbers reached high values (40.06) by the 20th day of storage. This appears to correlate with organoleptic results. However, volatile acid production in the fillets irradiated to 0.6 megarad did not show any significant increases throughout the storage test.

Conclusions

The shelf life of yellow perch fillets stored at 42°F is approximately 6 days. Irradiation to 0.3 and 0.6 megarad provides a 3- and 4-fold increase, respectively.

Table 4. — Total volatile acids (TVA) content of yellow perch irradiated at 0.0, 0.3, and 0.6 Mr, and stored at 42°F.

Days in Storage	TVA (VAN)		
	0.0 Mr	0.3 Mr	0.6 Mr
1	5.23	0.27	4.09
3	2.62	0.0	1.34
6	30.70	2.55	4.63
8	51.61	1.27	2.01
10	---	2.95	4.09
13	---	3.62	0.0
15	---	7.85	0.20
20	---	40.06	3.15
24	---	93.67	3.35

Re-Evaluation of Irradiated Yellow Perch
Fillets Stored at 33°F

Objective

To determine the maximum shelf life of yellow perch fillets irradiated to 0.0, 0.3, 0.6 megarad and stored at 33°F.

Materials

Fifty pounds of yellow perch fillets hand-cut from Lake Michigan gillnetted fish (South Haven, Mich., September 1964).

Experimental Procedures

Sensory Evaluation: Preparation, cookery, and presentation of samples remained the same as previously reported except that the frozen reference samples were not irradiated.

Microbiological Examination: The procedures remained the same as previously reported.

Chemical Tests: None conducted.

Results and Discussion

Sensory Evaluation: Table 5 presents the sensory scores for overall acceptability. Non-irradiated samples were unacceptable between the 8th and 13th day of storage. Fillets irradiated to 0.3 megarad were unacceptable between the 26th and 33rd day. Little difference was indicated by the sensory panel between fillets irradiated to 0.3 and 0.6 megarad. This may have been due to the use of nonirradiated reference samples.

Table 5. —Sensory scores for overall acceptability.

Days in Storage.	Irradiation dose (megarads)		
	0.0	0.3	0.6
6	7.6	-	-
8	7.1	6.8	6.3
13	2.9	6.4	-
14		-	6.0
19		6.5	-
20		-	6.3
26		5.0	4.8
33		4.5	3.8
42		3.8	4.3

Microbiological Examinations: Table 6 illustrates the variations in total aerobic microbial plate counts, and total yeast and mold plate counts of the unirradiated and nonirradiated yellow perch fillets stored at 33°F.

The initial microbial count (20°C) of the unirradiated samples averaged approximately 8.62×10^5 . By the 5th day of storage the sample had reached a total count of 4.4×10^7 .

Irradiation to 0.3 megarad reduced the total microbial count by 99.9 percent. By the 22nd day of storage it reached a count of 1.32×10^7 .

Irradiation to 0.6 megarad reduced the total microbial count by nearly 100 percent. By the 50th day of storage it reached a count of 1.07×10^7 .

Yeast and mold counts were reduced by both irradiation levels and remained at insignificant numbers throughout subsequent storage. Yeast and mold counts in the 0.6 megarad samples were not obtained until the 40th day of storage. It is interesting to note that the yeasts and molds were more sensitive to irradiation than the ones present in our 33°F storage test conducted during the same season the previous year (September 1963). At that time, we reported yeasts and molds as the principal cause of spoilage in the irradiated samples. Otherwise, the pattern of the gross flora growth curves in the unirradiated and irradiated samples is similar.

Conclusions

The data obtained from this test is generally similar to that obtained from a similar test conducted one year ago (Annual Report to the AEC, 1964) with the exception of the sensory scores for samples irradiated to 0.6 megarad.

Table 6. — Total aerobic microbial plate counts of unirradiated and irradiated yellow perch fillets stored at 33°F.

Storage time (days)	0.0 Mrad				0.3 Mrad				0.6 Mrad			
	Microbial counts		Yeast and molds		Microbial counts		Yeast and molds		Microbial counts		Yeast and molds	
	per gram		per gram		per gram		per gram		per gram		per gram	
	20°C	3°C	20°C	3°C	20°C	3°C	20°C	3°C	20°C	3°C	20°C	3°C
	9×10^5	5.32×10^5	5.16×10^3	5.64×10^3								
	8.86×10^5	5.96×10^5	6.96×10^3	5.64×10^3								
	8.0×10^5	4.28×10^5	4.34×10^3	4.32×10^3								
	av:	av:	av:	av:								
0	8.62×10^5	5.18×10^5	5.48×10^3	5.2×10^3								
1					0.96×10^3	0.36×10^3	0.24×10^3	---	1.56×10^3	---	---	---
4	7.64×10^6	6.32×10^6	2.66×10^4	2.4×10^4								
5	4.4×10^7	4.8×10^7	6.4×10^3	6×10^3								
8					1.33×10^3	0.48×10^3	0.16×10^3	---	1.2×10^3	---	---	---
12					5.16×10^3	---	---	---	0.4×10^3	---	---	---
15					2.87×10^4	3.46×10^4	0.48×10^3	5.52×10^3	---	---	---	---
19					1.56×10^5	1.4×10^5	3.96×10^3	5.88×10^3	0.36×10^3	---	---	---
22					1.08×10^6	9.6×10^5	5.04×10^3	5.76×10^3	4.44×10^3	4.4×10^3	---	---
26					1.32×10^7	1.44×10^7	$>10^3$	$>10^3$	$.48 \times 10^3$	1.26×10^3	---	---
29					2.8×10^6	2.8×10^6	4.4×10^4	3.2×10^4	2.09×10^4	2.16×10^4	$.24 \times 10^3$	0.24×10^3
33					5.88×10^7	6.92×10^7	6.68×10^5	7.48×10^5	1.68×10^3	2.8×10^3	---	---
36					9.2×10^6	9.6×10^6	2.8×10^4	4.4×10^4	3.64×10^4	5.72×10^4	---	---
40					7.6×10^6	6.4×10^6	2.16×10^4	1.88×10^4				
43					1.4×10^7	1.72×10^7	4×10^4		7.16×10^6	7.52×10^6	1.85×10^4	9.36×10^3
47					3.16×10^7	3×10^7	6.44×10^4	7.24×10^4	7.76×10^6	6.28×10^6	3.38×10^4	3.48×10^4
50									2.66×10^6	2.52×10^6	8×10^3	7.2×10^3
54									1.07×10^7	1.12×10^7	2.6×10^4	2.08×10^4
61									1.8×10^7	1.8×10^7	2.72×10^4	2.4×10^4
									$1. \times 10^7$	9.4×10^6	2.88×10^4	2.48×10^4

* No growth observed.

2. Smoked Chubs

Because of the current processing and holding restrictions imposed by the states bordering the Great Lakes as a result of the 1963 botulism outbreaks involving smoked fish, it has become apparent that the application of gamma radiation as a means of extending the refrigerated shelf life in sensory terms only would now have little or no practical application to this product. However, studies of the possible use of the irradiation process as an adjunct to other processing parameters such as time and temperature of heating, salt content, the use of bacteriostatic additives, etc., could result in information of real value to the industry and the consuming public.

At present, the smoked fish industry is experiencing great difficulty in producing a product whose processing conditions conform to state regulations and whose eating quality is acceptable to the consumer. State regulations require that the fish be heated to an internal temperature of 180°F and this temperature be held for 30 minutes. This temperature greatly exceeds that of the traditional process. The use of irradiation might allow for a reduction in the required heating conditions. The possibility that such application might be successful has been indicated by the findings of the Army's Natick Laboratories in that relatively high doses of irradiation can be applied to raw beef products while frozen, resulting in only minor induction of irradiation flavors/odors.

Thus, preliminary studies were initiated at this laboratory to determine if irradiation could be applied to the processing of smoked fish to increase its saleability without detriment to that level of consumer safety attained through the use of current time-temperature processing requirements. These studies were necessarily preliminary in nature and it is anticipated that they will be continued in further depth during the next contract year.

Effect of Brining Time and Brine Strength on Salt Content of Smoked Chubs Produced from Frozen Stocks

Objective

To determine the effect of brining time and brine concentration on the salt content of smoked product from chubs held in storage at 0°F.

Materials

100 pounds of 60° salometer brine, and 80 pounds of medium-size raw frozen chub (gutted, head-on).

Procedure

The initial brine stock was prepared by dissolving 15.6 pounds of salt (NaCl) in 84.4 pounds of tap water (at 60°F). From this, brines of 45°, 30°, and 15° salometer were obtained by

appropriate dilutions. Twenty pounds of brine at each strength were placed in a 33°F refrigerator to attain storage temperature (33°F) prior to brining of fish.

Ten pounds of thawed chubs were placed in the brine to give an initial brine to fish ratio of 2:1. Samples were removed from the brines according to the following schedule.

Sample Removal Schedule			
Brine strength (° salometer)			
<u>15°</u>	<u>30°</u>	<u>45°</u>	<u>60°</u>
1 hour	1 hour	---	---
9 hours	9 hours	9 hours	---
---	24 hours	24 hours	24 hours
---	---	---	42 hours

After brining, one portion of each sample was analyzed for percentage salt, and another portion was smoked to 180°F internal for 30 minutes and then analyzed for salt. Samples for analysis consisted of the skin-on fillet for the brined-only fish, and the dorsal muscle (skin removed) for the smoked fish. Percentage salt was determined according to the Official Methods of Analysis, AOAC, 9th Ed.

Results and Discussion

Table 7 presents the percentage of salt found in raw, brined fish and in finished product from the various brining treatments. Generally, as brining time or brine strength increased, so also did salt content.

Table 7.—Effect of brine strength and brining time of salt content of brined and smoked fish from frozen stocks.

Brining treatment (Salometer/time)	Percentage NaCl	
	Brined-only fish	Smoked fish
15° - 1 hr	0.60	0.82
15° - 8 hr	1.32	1.41
30° - 1 hr	0.93	1.61
30° - 8 hr	1.75	2.06
30° - 24 hr	3.48	3.57
45° - 8 hr	2.18	3.29
45° - 24 hr	4.06	4.29
60° - 24 hr	3.62	4.04
60° - 42 hr	4.43	5.99

Effect of Irradiating Temperature
On Irradiation Flavor Development—Test 1

Objective

To determine the effect upon irradiation flavor development of irradiation of raw fish at either 0° or 33°F at irradiation doses of either 2.0 or 4.5 megarads.

Procedures

All fish were from the same lot, which had been frozen 2 days after capture and held at 0°F until this test was conducted. A portion of the fish was thawed and held at 33°F, during irradiation. The actual dose rates under which the samples were irradiated were 200 Krad/hr for 21.7 hr = 4.34 megarad (high dose samples) and 100 Krad/hr for 21.7 hr = 2.17 megarad (lower dose samples). Another portion was irradiated while in the frozen state. A third portion was thawed and used as a control. After irradiation treatment, all samples were brined in a 25° salometer brine for 18 hours, and then smoked to 150°F internal for 30 minutes. After smoking, the loin muscles were removed, flaked and mixed for presentation to a 4-member sensory panel. Samples were scored for flavor according to a 9-point hedonic scale.

Results

Table 8 presents the results of sensory evaluation of smoked fish samples processed under the 2 irradiating temperatures and to the 2 dose levels. Since a score of 5 or higher must be obtained for acceptable eating quality, it can be seen that, of the treated samples, only those irradiated in the frozen state at these relatively high doses were considered to be acceptable.

Table 8.—Sensory evaluation of smoked chubs irradiated at 0° and 33°F to levels of 2.17 and 4.34 megarads.

Sensory Scores					
	Controls	2.17 Mrad		4.34 Mrad	
		33°F	0°F	33°F	0°F
	7	4	8	4	6
	7	5	7	5	5
	9	6	8	6	8
	8	4	3	3	4
Average	7.8	4.8	6.5	4.5	5.8

Conclusions

Irradiation of raw product in the frozen condition appears to markedly reduce irradiation flavor in smoked chub. High levels of irradiation can be tolerated.

Effect of Irradiating Temperature on
Irradiation Flavor Development — Test 2

Objective

To determine the effect on irradiation flavor development in raw chub irradiated at 0°F to dose levels ranging from 0.3 to 1.4 megarads.

Procedure

Four lots of raw, dressed chub were irradiated while frozen at 0°F to 4 dose levels: 0.343, 0.679, 1.028, and 1.461 megarads. After irradiation, the fish were thawed, brined at 35° salometer for 18 hours, and smoked to 180°F for 30 minutes. Nonirradiated controls were thawed, brined, and smoked collectively with treated samples.

After smoking, the loin muscles of the fish from each lot were removed, flaked, and mixed together to provide a reasonably uniform mixture of smoked fish flesh for each treatment. A 5-member sensory panel evaluated the samples for odor, flavor, and overall acceptability. Three samples were presented per session consisting of either 1 control and 2 samples irradiated at the same dose, or 2 controls and 1 treated sample. The panelists were requested to identify the unlike sample and then to evaluate each of the samples using a 9-point hedonic scoring system (a score of 5 or higher indicated acceptance of the product).

Results and Discussion

Table 9 presents the sensory scores obtained on the smoked fish flesh treated in the above manner. The average score for reference samples was 8.2, closely followed by an average score of 7.8 for fish irradiated to 0.343 megarad. Scores for fish irradiated to 0.679-1.461 megarads ranged from an average of 6.4 to 7.0.

Table 9.—Sensory evaluation of smoked chub produced from raw fish irradiated at 0°F to levels ranging from 0.343 to 1.370 megarads.

<u>Panel Member</u>	<u>Reference</u>	<u>0.343 Mrad</u>	<u>0.679 Mrad</u>	<u>1.028 Mrad</u>	<u>1.370 Mrad</u>
1	8	7	7	8	7
2	8	9	7	8	4
3	8	8	7	6	7
4	9	7	4	4	7
5	8	7	7	8	8
Average	<u>8.2</u>	<u>7.8</u>	<u>6.4</u>	<u>7.0</u>	<u>6.6</u>

Thus, although those fish receiving the lowest dose were considered to be most similar to the reference samples in flavor, those fish irradiated at the higher levels were all found to be acceptable.

REFERENCE

Emerson, J. A., N. Kazanas, R. A. Greig, H. L. Seagran, P. Markakis, R. C. Nicholas, B. S. Schweigert, and L. L. Kempe, 1964. Irradiation preservation of fresh water fish and inland fruits and vegetables. Prepared for the Division of Isotopes Development, U.S.A.E.C. Report number COO 1283-12, Isotopes-Industrial Technology, TID 4500 (19th Ed.).

PART B. MICROBIOLOGY OF YELLOW PERCH

1. Isolation and Identification

Studies on the generic identification of bacteria isolated from nonirradiated and irradiated perch samples during the general course of microbial spoilage is reported here. In addition, total yeast and mold counts from these samples are discussed. A detailed knowledge of the processing technique on these flora, is important in establishing irradiation dose level and development of more meaningful criteria to estimate quality loss.

Little work has been reported concerning the microflora of freshwater fishery products of the United States. Bogoslawski (1961) published an abstract on freshwater fish bacteria, however, his findings have not been published in detail. Evelyn and McDermott (1961) reported studies on the microflora of several species of freshwater fish from Ontario — trout species accounted for 97.1 percent of the fish examined; the remainder were "coarse" fish.

The current report presents microbiological data on commercially processed fillets which contained the commensal plus contaminating flora. To our knowledge, this is the first study reported on fresh and spoiling nonirradiated and irradiated freshwater fishery products. It is hoped that the identification of these microflora will complement our previous and current studies on the pattern and significance of gross flora change in relation to quality of untreated and irradiation treated yellow perch fillets.

Methods

Bacteria: Following the enumeration and tabulation of macrocolonies on petri dish culture plates obtained from perch fillets during the general course of microbial spoilage occurring at temperatures of either 33° or 42°F (Emerson et al., 1964); representative colonies from TPY-extract agar plates were selected and keyed out as to generic designation. For this purpose, the isolates were screened into groups employing essentially the morphological and biochemical protocols of Shewan (1960 a) and Masurovsky (1963) to facilitate their grouping as to genus. Our modification of the aforementioned schemes is presented in Figure 1. The isolates were initially screened into groups, then taking one group at a time, their identification was further confirmed by referring to Bergy's Manual (7th Ed. 1957).

FIGURE 1. GENERIC IDENTIFICATION SCHEME FOR BACTERIA ISOLATED FROM PERCH FILLETS

Gram positive organisms have been reported to be inhibited by the dyes in MacConkey's medium (Difco Manual). Because gram positive organisms such as Micrococcus, Corynebacterium and Brevibacterium have been reported to be readily decolorized (Bergey's Manual), all isolates were plated on MacConkey's medium. Inhibition of organisms on this medium plus other morphological and biochemical characteristics helped to identify those gram positive organisms easily decolorized and giving a false gram negative stain.

The data obtained from each isolate was coded and recorded on IBM cards, which through mechanical sorting has further aided these bacterial identifications. These cards constitute a versatile and permanent record in our culture documentation.

Yeasts: The procedure employed for total yeast counts is the same as described in the previous annual report (Emerson et al., 1964).

Results

Bacteria: The total number of organisms of each genus found in the nonirradiated and irradiated fresh and spoiling perch fillets stored at either 33° or 42°F are presented in Table 10. This serves to indicate the gross composition of the bacterial flora found in the products during the entire period of storage. Those organisms found in greatest numbers in the overall sense were Pseudomonas, Micrococcus, Achromobacter, Alcaligenes and Brevibacterium.

Figure 2 presents the average total microbial counts for all tests as determined during the course of storage. Also indicated are those organisms isolated at various times of storage. It can be seen that nonirradiated samples had an initial count of approximately 6×10^5 , whereas, the initial counts of samples irradiated at 0.3 and 0.6 megarad were approximately 4×10^3 and 2×10^3 , respectively. No lag phase was noted in the nonirradiated samples, whereas, a lag period of about 6 and 18 days of storage were observed for those samples irradiated at 0.3 and 0.6 megarad, respectively. Average growth rates for all samples appeared to be the same. It was found that the counts entering the stationary phase were approximately 1×10^8 for the control samples, whereas, the counts of samples irradiated at 0.3 and 0.6 were approximately 1 log number lower.

Organisms found in the nonirradiated perch on initial isolation, in order of decreasing number, were: Pseudomonas, Micrococcus, Brevibacterium, Achromobacter, Alcaligenes, Bacillus, Sarcina, Corynebacterium, Flavobacterium and a few Proteus, Escherichia, and Lactobacillus. A few unidentified isolates resembling mycobacteria (fast growers) and microbacteria were found and will be further investigated. As spoilage progressed, the flora shifted and the pseudomonads became predominant. At the 4th day of storage, there were still a few pigmented bacteria (Micrococcus and Brevibacterium) isolated from the samples, but none were isolated after

Organisms: PS-Pseudomonas, M-Micrococci, Br-Brevibacterium, AA-Achr-alcalig, B-Bacillus, S-Sarcina, C-Corynebacterium, F-Flavobacterium, P-Proteus, E-Escherichia, L-Lactobacillus, Y-Yeast.

FIGURE 2. EFFECT OF IRRADIATION DOSAGE ON GROWTH RATE AND LAG PHASE OF TOTAL MICROBIAL PLATE COUNTS FROM FILLETS STORED AT 33°F, AND ORGANISMS ISOLATED AT DIFFERENT STORAGE TIMES

the 5th day. Micrococci were still present by the 12th day of storage, although the pseudomonads were predominant (Pseudomonas species have been reported to be principally responsible for the spoilage of marine fish).

Irradiation of samples to 0.3 and 0.6 megarad eliminated the most predominant genus, Pseudomonas. A few brevibacteria, micrococci, and yeasts survived these doses, but the predominant organisms isolated throughout the storage period were of the achromobacter-alcaligenes group.

Yeasts: Figure 3 illustrates variations in total yeast plate counts of unirradiated and irradiated perch fillets stored at 33°F. Yeast counts in nonirradiated samples remained at insignificant levels throughout storage. Similar results were observed for irradiated samples, except in one storage test where both the 0.3 and 0.6 megarad samples showed a high level of yeast growth. The flora of these samples exhibited a shorter lag phase than those from Lake Michigan. Sour and yeasty odors were produced from these surviving yeasts. It is interesting to note that the fish used in this particular test came from Lake Erie, whereas, the others came from Lake Michigan. However, the initial yeast contamination was higher in those samples from Lake Michigan as against those from Lake Erie. No explanation for the high yeast outgrowth in irradiated Lake Erie samples is available at this time.

Table 10. — Total number of organisms of each genus isolated from irradiated and nonirradiated perch fillets stored at 33° and 42°F.

Organisms isolated (Genus)	Storage temperature					
	33°F			42°F		
	Dose (Mr)			Dose (Mr)		
	0.0	0.3	0.6	0.0	0.3	0.6
Pseudomonas	64	0	0	9	4	0
Micrococcus	26	0	0	5	0	0
Achr-Alcalig	20	39	10	3	13	5
Brevibacterium	21	1	1	1	0	0
Sarcina	6	0	0	2	0	0
Corynebacterium	4	0	0	2	0	0
Bacillus	8	0	0	2	1	0
Escherichia	1	0	0	0	0	0
Proteus	3	0	0	0	0	0
Flavobacterium	3	0	0	0	0	0
Lactobacillus	1	0	0	0	0	0
Yeast	0	7	2	1	0	0

2. Proteolytic Activity of Bacterial Isolates

Organisms from spoiling fish, which grow at low temperatures and exhibit proteolytic activity, have been assumed to be primarily responsible for spoilage (Shewan, Hobbs, and Hodgkiss, 1960b).

FIGURE 3. TOTAL YEAST PLATE COUNTS FROM IRRADIATED AND NONIRRADIATED PERCH FILLETS OF THREE STORAGE TESTS (Sept. 1963, Feb. 1964, Sept. 1964) AT 33°F

In order to evaluate the proteolytic activity of organisms isolated from yellow perch, a fish substrate which would be readily available and could be easily sterilized was developed. To preserve the native state of the heat labile constituents, cold sterilization was necessary. Although filtration-sterilization could accomplish cold sterilization, the various difficulties that would be involved removed this method from consideration. Thus, a raw perch-extract was sterilized through the use of irradiation and then combined with sterile agar to provide the final medium. Preliminary results showed that this medium could be used as a suitable experimental substrate for the study of bacterial proteolytic activity during fish muscle spoilage.

During the general course of microbial spoilage, irradiated and nonirradiated bacteria which were isolated and identified were also screened for proteolytic activity on sterile, raw perch-extract agar medium and confirmed using the conventional caseinolytic activity test on a skim milk agar.

Materials

Perch-extract agar medium: Perch was frozen immediately after catch, then dressed and skinned. The flesh was ground and pressed. For the latter operation, a Carver laboratory press, Model B was utilized. The pressed juice was passed through a double thickness of Whatman No. 40 filter paper on a Büchner funnel. The pressed juice was then sterilized by Co⁶⁰ gamma radiation to a dose of 3.0 megarads while being held at 33°F. Incubation of this extract at 20° and 30°C for as long as a month showed no microbial growth. Fish-extract agar plates were prepared by adding 100 ml of the sterile fish-extract to 1 liter of sterile 1.5 percent agar, holding both ingredients at 42°C prior to mixing.

30 percent skim milk medium: Commercially prepared sterile evaporated skimmed milk concentrated 2-fold was employed. A 30 percent skim milk agar medium was prepared by adding 15 cc of the skimmed milk to 85 cc of sterile nutrient agar (0.3 percent bacto-beef-extract, 0.5 percent bacto-peptone, and 1.5 percent agar) held at 42°C and poured into petri dishes.

Procedure

Isolates were streaked on the perch-extract agar, and on skim milk agar and incubated at 20°C for 24 to 48 hours. After this period, plates were reincubated at 30°C, showing this temperature to be more suitable for proteolytic activity of some organisms. Optimum growth temperature for all isolates was found to be between 20° and 30°C. Caseinase or fish muscle proteases could be adaptive enzymes, being elaborated only as needed, thus stimulation of these extracellular enzymes sometimes happens under repeated transfer to fresh substrate. Therefore, those isolates (after primary isolation from TPY medium) showing negative activity in either milk or fish-extract agar were retransferred to fresh milk or fish-extract agar, respectively, which could finally result in the utilization of the substrate. Organisms showed proteolytic activity on these media by a clearing zone in the medium surrounding the isolate.

Results

Table 11 shows the number of proteolytic bacteria isolated from nonirradiated and irradiated perch. Proteolytic activity on skim milk and perch-extract agar was demonstrated by some of the pseudomonads, brevibacteria, micrococci, sarcina, corynebacteria, and yeasts. Most of the organisms showing proteolytic activity were those isolated from nonirradiated perch—particularly the pseudomonads which became predominant while spoilage progressed. Of the 38 pseudomonads tested, 33 showed proteolytic activity. Irradiation to 0.3 and 0.6 megarad eliminated essentially all proteolytic bacteria. The only irradiated organism isolated from perch stored at 33°F showing proteolytic activity on both media was an unidentified pink yeast isolated in TPY immediately after irradiation and during the first 8 days of sample storage. The yeasts isolated on malt agar were not tested for proteolytic activity on either media.

The irradiated fillets stored at 42°F showed few pseudomonads, bacillis, and brevibacteria surviving irradiation, but they were no longer evident by the 3rd day of fillet storage.

All isolates plated on the irradiation-sterilized perch extract medium exhibited good growth in 24 hours. Of those organisms showing proteolytic activity, most exhibited this characteristic on both media. However, a few showed proteolytic activity either on the perch extract or on the skim milk agar. Of a total of 233 isolates tested, 54 were found to be positive for proteolytic activity. Of these, 42 were positive on both media, an additional 8 were positive only on the skim milk, and 4 others were positive only on the perch extract.

Table 11.—Proteolytic activity of isolates from irradiated and nonirradiated yellow perch fillets stored at 33° and 42°F.

Storage temp. (0°)	Dose (Mrad)	Number isolated ¹	Total	Pigm.	Non-Pigm.	Pseudomonas	Brevibacterium	Micrococcus	Sarcina	Bacillus	Corynebacterium	Yeast	Unidentified	Lost
33	0.0	79	32	20	12	18	6	4	1	0	1	0	1	1
	0.3	53	6	6	0	0	0	0	0	0	0	6	0	0
	0.6	39	2	2	0	0	0	0	0	0	0	2	0	0
42	0.0	30	8	5	3	1	0	3	2	0	1	1	0	0
	0.3	23	4	1	3	3	0	0	0	1	0	0	0	0
	0.6	9	2	1	1	0	1	0	0	1	0	0	0	0
Totals		233	54	35	19	22	7	7	3	2	2	9	1	1

¹Yeast initially isolated on malt agar are not included in this table.

Conservation of Bacterial Isolates

Several excellent methods are available for preserving microorganisms (Martin, 1964) and with many organisms there is a choice of at least two proven techniques. Lyophilization is the method most commonly used for long-term conservation. The advantages of lyophilization, especially in the maintenance of large numbers of cultures, are considerable (Haynes, 1955 and 1963). Several factors have been shown to influence the survival of microorganisms in the lyophilized state (Fry, 1954; Greaves, 1960).

In the current investigations, bacterial isolates from irradiated and nonirradiated yellow perch fillets were lyophilized and stored following their identification.

Materials

The lyophilizing menstruum employed was "mist dessicans" (Fry and Greaves, 1951; Fry, 1954) prepared with 3 parts serum and 1 part peptone broth plus 10 percent glucose. The serum was employed to form a "solid dry cake." Glucose was added as a buffer to hold the residual moisture at about 1 percent (Muggleton, 1963). Ten percent Difco-peptone and 10 percent glucose were mixed together after being sterilized and the mixture employed for suspending the cultures to be lyophilized. Bovine serum was prepared from whole blood obtained from a local abattoir and sterilized by Seitz filtration.

Procedure

Mature cultures at the maximum stationary phase were employed. When cultures were pigmented, harvesting was done after pigment was fully developed. To the mature culture, 2 cc of peptone-sugar mixture were added, the culture loosened with the pipette, and the organisms suspended. To a sterile ampule containing 0.8 cc of sterile bovine serum, 0.25 cc of the peptone-sugar cell mixture was added. The ampules were loosely plugged with cotton and freezing carried out in two stages. The suspension was first frozen in powdered dry ice, rotating the ampule to form a shell of the cell material—cooling to about -20°C . The ampule was then transferred to an ice-acetone mixture to rapidly lower the temperature to approximately -50°C (Muggleton, 1963). Cells were then lyophilized utilizing a Virtis "Bio-Dryer Unitrap" apparatus. Lyophilization of the bacterial preparations required about 3-4 hours, after which the ampules were sealed under vacuum. Lyophilized cultures were then stored at $3-5^{\circ}\text{C}$.

Conclusions

The pseudomonas, which were found to be greater in number in the nonirradiated product and the predominant toward spoilage of the fillets, were essentially eliminated by irradiation to 0.3 and 0.6 megarad. Irradiation to these levels eliminated the proteolytic bacteria, leaving a residual flora composed of the achromobacter-alcaligenes group.

Preliminary results showed that perch-extract agar could be used as a suitable experimental substrate for the study of microbial proteolytic activity during fish muscle spoilage.

REFERENCES

- Bergy's Manual of Determinative Bacteriology, 1957. 7th Ed. Williams and Wilkins Co., Baltimore, Maryland.
- Bogoslowski, A. S., 1961. Fisheries Research. IV. Fresh-water fish bacteria. Canadian Fisherman (Natl. Business publication, Ltd., Gardenvale (Que., Canada), 48(7):34(a).
- Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures. 1953. 9th Ed., Difco Laboratories, Inc., Detroit, Michigan.
- Duggan, D. E., A. W. Anderson and P. R. Elliker. 1963. Appl. Microbio., 11:398-403.
- Emerson, J. A., N. Kazanas, R. A. Greig, H. L. Seagran, P. Markakis, R. C. Nicholas, B. S. Schweigert and L. L. Kempe. 1964. Irradiation preservation of fresh water fish and inland fruits and vegetables. Prepared for the Division of Isotopes Development, U.S.A.E.C. Report number COO 1283-12, Isotopes-Industrial Technology, TID4500(19th Ed.).
- Evelyn, T. P. T., and L. A. McDermott. 1961. Bacterial studies of fresh water fish. I. Isolation of aerobic bacteria from several species of Ontario fish. Can. J. of Microbio (National Research Council, Ottawa, Canada), 7:375-82.
- Fry, R. M. 1954. The Preservation of Bacteria in Biological Appl. of Freeze and Drying, Ed. R. J. C. Harris, N. Y., Academic Press.
- Fry, R. M., and R. I. N. Greaves, 1951. J. Hyg., 49:220-46 (1951).
- Greaves, R. I. N. 1960. Recent Research in Freezing and Drying, Parkes, A. S., and Smith, A. U., Eds., Blackwell, Oxford.
- Haynes, W. C., L. J. Wickerham and C. W. Hesseltine. 1955. Appl. Microbiol., 3:361-68.
- Haynes, W. C. 1963. Culture Collections: Perspectives and Problems, Martin, S. M., Ed., University of Toronto Press, Toronto.
- Lerke, P., R. Adams, and L. Farber. 1963. Bacteriology of spoilage of fish muscle, Appl. Microbiol., 11:458-462.
- Martin, S. M. 1964. Conservation of microorganisms. Ann. Rev. Microbiol. 18:1-16.
- Masurovsky, E. B., J. S. Voss and S. A. Goldblith. 1963. Changes in the microflora of haddock fillets and shucked soft shelled clams after irradiation with Co⁶⁰ gamma rays and storage at 0°C and 6°C. Appl. Microbiol. 11(3):229-238.

Muggleton, P. W. 1963. Progr. Ind. Microbiol., 4:191-214.

Shewan, J. M., G. Hobbs, and W. Hodgkiss. 1960 a. A determinative scheme for the identification of certain genera of gram-negative bacteria, with special reference to the pseudomonada-ceal. J. Appl. Bact., 23(3):379-390.

Shewan, J. M., G. Hobbs and W. Hodgkiss. 1960 b. The Pseudomonas and Achromabacter groups of bacteria in the spoilage of marine whitefish. J. Appl. Bact., 23:463-468.

PART C. THE EFFECT OF GAMMA RADIATION ON ANTIMETABOLITES CONTAINED IN FISH

Introduction

Thiaminase, an antithiamine factor indigenous to certain species of fish, is undesirable in food and feed products made from fish containing this antimetabolite. Due to the current interest and possible use of irradiation in processing industrial fishery products, it is important to know the effect of gamma radiation on this enzyme.

The thiaminase-active fish that are currently being used for animal foods are usually processed by cooking at high temperatures to destroy the antithiamine factor. Cooking alone, however, is often detrimental to the acceptability and nutritive quality of the product. The simultaneous use of gamma radiation and mild heating, or even high levels of radiation alone, could likely serve as alternate approaches to inactivate the enzyme without thoroughly cooking the product.

Initial tests at this laboratory showed that the thiaminase of alewife viscera could be destroyed by gamma radiation. In view of these results, investigations were started under this contract to study more thoroughly the effect of radiation on this enzyme. The principal objective of this study was to determine the radiosensitivity of thiaminase in situ in various species of fish under controlled conditions of pH and temperature.

Methodology

Radiation processing of industrial fishery products would most likely be done on whole fish and whole fish wastes. Thus, the irradiation was carried out in such a manner as to simulate industrial conditions. Following irradiation, samples were assayed for residual thiaminase activity by standardized procedures.

Preparation of samples: Fresh whole fish were ground once through a 1/4-in. plate, mixed, and then ground once through a 1/8-in. plate. The ground material was then packed and sealed in metal TDT cans (about 20 grams capacity), and held at 0°C until irradiated. These cans

proved superior to plastic bottles that were used initially. Control of sample size and homogeneity, which is an important factor in these comparative studies, was thus improved.

Irradiation of samples: The canned fish samples (contained in an insulated cylinder) were placed in the center well of the cobalt-60 source. Temperature control during irradiation was accomplished by packing either dry ice or crushed ice above and below the samples in the insulated cylinder. Room temperature irradiation was carried out at ambient temperature of the source. Irradiation was done at the Phoenix Memorial Laboratory located on the campus of The University of Michigan.

Analysis of samples: Thiaminase activity of all the samples was determined by the assay procedure of Gnaedinger (1965). This procedure consists of first blending a known amount (5-10 grams) of thiaminase-active fish in a buffered KCl solution. The homogenate is then mixed with a known amount of thiamine hydrochloride. Enzyme activity is then determined by following, fluorometrically, the rate of thiamine destruction. Thiaminase activity curves are shown in Figures 4 and 5, relating effect of dosage level to residual activity at irradiation temperatures of 33° and 90°F, respectively.

Investigations

In Table 12 are given the variables that were studied under this project (species, pH, and temperature). Various species were used in order to determine whether differences existed between species in the radiosensitivities of their respective thiaminases. Since slight differences in natural pH were noted between species, one test was conducted to determine the effect of this variable on samples of whole alewife that were adjusted to pH 8.1 and 4.6 with NaOH and HCl, respectively. The combined effect of irradiation and temperature was also studied.

Summary of Results

Thiaminase activity of whole fish was not reduced when samples of whole alewife, carp, and shad were packed in dry ice and irradiated to 6 megarads; however, there are indications that alewife at pH 8.1, whole smelt, and spottail subjected to the same conditions are slightly radiosensitive.

At 33°F, up to 50 percent reduction occurred in some samples when irradiation was carried up to 4 megarads.

Still greater reductions were obtained when the samples were irradiated at room temperature and up to 4 megarads.

Changes in pH, above and below the normal pH, did not increase the radiosensitivity of thiaminase. In fact, there appeared to be a slight protective effect.

FIGURE 4. EFFECT OF GAMMA RADIATION ON THIAMINASE ACTIVITY OF WHOLE ALEWIFE, pH 6.90, 33°F

FIGURE 5. EFFECT OF GAMMA RADIATION ON THIAMINASE ACTIVITY OF WHOLE ALEWIFE, pH 6.90, 85°-95°F

Results indicate that the thiaminases of different species may not be equally sensitive to gamma radiation.

Table 12. — The effect of gamma radiation on fish thiaminase.

Species	pH	Dosage (megarads)	Observed reduction in thiaminase activity ²		
			<0° F ³	33° F	85°-95° F
Alewife	6.90 ¹	6	0	-	-
Carp	6.75 ¹	6	0	-	-
Shad	6.65 ¹	6	0	-	-
Smelt	6.85 ¹	6	Slight reduction	-	-
Shiner	6.70 ¹	6	"	-	-
Alewife	4.6	4	0	20	-
Alewife	8.1	4	Slight reduction	26	-
Alewife	6.90	4	-	56	80
Shad	6.65	4	-	13	43
Smelt	6.85	4	-	52	95

¹Natural pH.

²Expressed as a percentage of the activity of unirradiated control samples.

³Samples irradiated in dry ice.

Discussion

The principal conclusions drawn from this study are: (1) radiation has no apparent effect on thiaminase when irradiated in situ in the frozen state, and (2) the combined use of temperature and radiation shows the most promise for possible adaptation to commercial processing of industrial fish. The proposed next year's study includes a detailed examination of the latter relationship, which will involve temperatures up to 180° F.

Two other interesting observations were made, both of which could warrant additional study. First, there appeared to be differences in the thiaminases of different species, judging from their different radiosensitivities. The general composition of the fish or the concentration of activators or inhibitors could, however, account for this variation. Secondly, the apparent protective effect of pH levels above and below the natural pH is at present unexplained. Further study in this area, using purified enzyme systems, will be necessary.

REFERENCE

Gnaedinger, R. H. Thiaminase activity of fish: An improved assay method. Fishery Industrial Research (In press).

PART D. PUBLICATIONS AND PRESENTATIONS

Effect of gamma irradiation on the microflora of fresh water fish. I. Microbial load, lag period and rate of growth on yellow perch (Perca flavescens). Nuria Kazanas, J. A. Emerson, H. L. Seagran, and L. L. Kempe. (In preparation.) To be submitted to Food Technology.

Irradiation preservation of fresh water fish. I. Extension of refrigerated storage life of fresh yellow perch fillets. J. A. Emerson, Nuria Kazanas, R. A. Greig, H. L. Seagran, and L. L. Kempe. (In preparation.) To be submitted to Food Technology.

Effect of gamma irradiation on the microflora of yellow perch fillets. Nuria Kazanas. Presented January 9, 1965, at the Winter Meeting of the Michigan Branch of the American Society for Microbiologists, Wayne State University, Detroit; at the Eighth Conference on Great Lakes Research, Ann Arbor, March 29-30, 1965; and at the Sixteenth Annual Meeting of the Pacific Fisheries Technologists, Sidney, B. C., Canada, March 21-24, 1965.

Microbiological studies on irradiated yellow perch (Perca flavescens) fillets — gross population changes and isolation of aerobic bacteria. Nuria Kazanas. Presented at the 65th Annual Meeting of the American Society for Microbiologists, Atlantic City, April 25-29, 1965.

Irradiation preservation of fresh water fish — Irradiation methodology, product parameters, and sensory approach. J. A. Emerson. Presented at the Eighth Conference on Great Lakes Research, Ann Arbor, March 29-30, 1965.

Section 2

IRRADIATION PRESERVATION OF FRUITS AND VEGETABLES

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SUMMARY

1. Gamma irradiation (200 krad) of strawberries, var. Robinson, will delay by 2-3 days the rapid decay caused by transportation without refrigeration and subsequent storage at 44°F.
2. Overwrapping with polyethylene film may add to the quality protection of Robinson strawberries afforded by 200 krad gamma irradiation.
3. This year no quality benefits were apparent on sweet cherries exposed to 150 krad gamma irradiation.
4. The dehydration of prunes is facilitated by gamma irradiation (200-400 krad).
5. Dry beans are softened by exposure to radiation up to 1.6 Mrad.
6. It is possible to reduce the yeast plus mold counts of apple juice to 0.2% of the original count by irradiation (250 krad) and storing the juice under anoxic condition.
7. An effort to extract more sugar from sugar beets by irradiation failed. However, some arresting of the browning of the shredded beets was observed.
8. The sprouting of Arenac and Ontario potatoes was inhibited by 7.5 krad of gamma irradiation.
9. The resistance to radiation of Aspergillus flavus spores, suspended in water, is rather low ($D = 45$ krad); the pH shows no effect, and glucose increases it slightly. Dry spores are more radioresistant than spores in ag. suspension.
10. Low dose irradiation (200-600 krad) releases calcium from plant tissues.

REPORT

During the second year of research under the AEC contract a number of experiments on the effect of irradiation on fruits and vegetables were carried out. The following is a rather condensed description of these experiments.

STRAWBERRIES

Two experiments on strawberry irradiation were carried out during the 1964 season. The objective of the first experiment was to see whether irradiation could allay the effect of transport on strawberries. The second experiment was aimed at studying overwrapping in combination with irradiation.

1. Shipment experiment. Robinson strawberries grown near Detroit were placed in 168 wooden pint boxes and transported to the nearby irradiation facility in cardboard shippers holding 12 pint boxes each. Overripe, under-ripe damaged fruit was excluded. For irradiation, 84 pint boxes were arranged in two 3-layer rings around the cobalt-60 source. The inner ring received 50 krad/hr and the outer ring 25 krad/hr. For uniform dose distribution, each box was turned by 180° around a vertical axis at half-dose time. The total dose was 200 krad. After

irradiation, the boxes were placed in the shippers, transported to the lab. in East Lansing, 60 miles away, and stored at 44°F. The control strawberries were handled similarly. The day following irradiation, the firmness of the irradiated and control fruit was tested by means of the Lee-Kramer shear press. For each testing 100 grams of fruit was placed in the cell. Figure 1 is a typical automatically recorded texture curve. The sharp peak is interpreted as indicating the shear-pressure, while the smoother part of the curve probably corresponds to the compression of the fruit. Seven such tests were performed for the irradiated berries and seven for the controls.

Two days after irradiation, all shippers were loaded on a station wagon and transported 234 miles to simulate commercial shipping of Michigan-grown strawberries. The fruit reached a temperature of about 80°F during transportation and it was placed in the 44°F room subsequently.

At intervals of time indicated in Table 1 the texture of irradiated and control fruit was tested exactly as it was done one day after irradiation. The results are summarized in Table 1. The irradiated fruit was generally softer, although the difference was not statistically significant on the 1st and 9th day.

Table 1.—Shear testing of control and irradiated (200 krad) strawberries at various times after irradiation. The numbers are peak Press readings and indicate maximum shear pressure.

Days After Irradiation	Control	Irradiated
1	52.8	43.3
3	62.3	47.7**
6	62.4	47.7**
8	65.1	53.9*
9	59.0	51.0

*5% probability level.

**1% probability level.

The effect of irradiation and shipping on the taste of the fruit was also tested with the participation of 15 tasters. Both the triangular and the pair comparison tests were used five times during the 15-day post-irradiation storage. No significant difference could be detected between irradiated and control strawberries.

The berries exhibiting lesions (mold growth or mushy spots) were also counted in samples of seven pints of fruit periodically and the results are presented in Figure 2.

This experiment indicates that 200 krad gamma irradiation of Robinson strawberries will delay the damage caused by transportation without refrigeration and subsequent storage at rather high temperature (44°F) by 2 to 3 days.

FIGURE 1. LEE-KRAMER SHEAR PRESS TRACING OF TWO 100-GRAM SAMPLES OF STRAWBERRIES

FIGURE 2. EFFECT OF GAMMA RAYS AND SHIPPING ON LESION APPEARANCE OF STRAWBERRIES

2. Overwrap experiment. Robinson strawberries grown near Detroit were exposed to 200 krad of gamma rays in pint boxes, at The University of Michigan irradiator. There were 4 groups of 156 boxes each. Two groups were irradiated, one of them with a 1.5 mil polyethylene wrap around each box, and the other without overwrap. Similarly, there were two groups of nonirradiated berries, one with and the other without an overwrap around each box.

After irradiation, the irradiated and control fruit was transported without refrigeration to Michigan State University, 66 miles away, and placed in a cooler at 42°-45°F. During storage at this temperature the following observations and tests were made.

Color. No color difference between irradiated and control berries was noticeable during the period of observation. This confirms previous results in which instrumental evaluation of color was used. There was more gloss on the strawberries in overwrapped boxes, both irradiated and control, than on those without the cover. Before unwrapping, however, the condensation on the inside of the film rendered the covered boxes somewhat less attractive.

Lesions. At periods of time indicated in Table 2, thirteen boxes were removed from cold storage, the berries in them were weighed and those with one or more visible lesions were removed and weighed in order to determine percent spoilage in weight. The spoilage judge, a person trained in food microbiology, did not know the identity of the fruit group she was working on. The results are presented in Table 2 and indicate that under the conditions of this experiment spoilage can be delayed 2 to 5 days by 200 krad gamma irradiation. Among the two non-irradiated fruit groups, that without overwrap suffered the greatest losses, while the irradiated and overwrapped fruit displayed the least spoilage of all four groups.

Table 2. — Effect of gamma irradiation (200 krad) and subsequent storage at 44°F on the spoilage of strawberries. Numbers indicate sound fruit on % weight basis (sample size, 13 pints).

Overwrapped			Non-overwrapped		
Days After Irradiation	Control	Irrad.	Days After Irradiation	Control	Irrad.
1	97.2	99.8	1	96.6	95.3
3	86.0	96.4	3	94.4	94.1
5	83.3	93.6	5	78.8	94.0
7	66.0	18.7	7	60.8	70.4
8	55.2	81.7	8	44.6	73.8
9	--	--	9	30.8	62.4
10	--	--	10	25.4	66.4
11	44.1	81.1	11	19.4	48.6
12	--	--	12	--	--
13	--	--	13	--	--
14	13.6	47.1	14	12.2	24.9
15	11.1	35.6	15	7.1	14.3
17	4.2	33.5	17	--	--
19	--	--	19	--	--
21	0	15.4	21	--	--

Texture. The firmness of the fruit was measured by means of the Lee-Kramer shear press at times indicated in Table 3. For each testing 100 grams of sound fruit were placed in the sample cell and 12 or 13 such tests were performed on each group every day of testing. The means of the maximum press readings were treated statistically and are presented in Table 3. Irradiation resulted in softening of the fruit and in spite of a remarkable recovery of firmness (about 50% in 13 days) the irradiated fruit remained softer than the control fruit throughout the storage period, since the control fruit also firmed up during storage.

Table 3. Effect of gamma irradiation and subsequent storage at 44°F on the texture of strawberries. Numbers indicate shear press peak readings (average of 12-13 tests).

Overwrapped				Non-overwrapped			
Days After Irradiation	Cont.	Irrad.	Diff.	Days After Irradiation	Cont.	Irrad.	Diff.
1	45.7	36.4	9.3**	1	48.2	35.7	12.5**
3	53.2	43.0	10.2**	3	51.8	37.8	14.0**
5	55.5	45.5	10.0**	5	59.8	44.2	15.6**
7	57.8	49.6	8.2**	7	59.1	46.6	12.5**
9	59.3	45.5	13.8**	9	60.5	47.6	12.9**
11	57.6	48.0	9.6**	11	60.2	52.6	7.6*
13	--	50.2	--	13	--	53.9	--
15	--	--	--	15	--	--	--
19	--	--	--	19	--	--	--

**1% probability level.

*5% probability level.

Taste. For taste comparisons 15 judges were used in a triangular test on the 1st, 3rd, and 7th day after irradiation. The berries were sliced and mixed with sugar at the ratio one sugar, ten fruit. No significant difference was found between unwrapped irradiated and unwrapped control berries. Wrapped control strawberries scored significantly lower than wrapped irradiated berries, after one day of post-irradiation storage. Apparently an off-flavor was developed in the wrapped control fruit.

This experiment indicates that overwrapping with thin polyethylene film may add to the quality protection of Robinson strawberries afforded by 200 krad gamma irradiation.

SWEET CHERRIES

Sweet cherries of the Schmidt variety, grown in western Michigan, were hand-picked, placed in wooden pint boxes and transported by automobile to the gamma irradiation on site at Ann Arbor, 240 miles away, without delay. There were four groups of fruit, each group composed of 156 pint boxes. Two groups were exposed to 150 krad irradiation, one of them with a 1.5 mil polyethylene wrap around each box, the other without overwrap. Similarly, there were two groups of nonirradiated cherries, one with the other without over wrap around each box.

After irradiation, all of the fruit was shipped to E. Lansing and stored at 38°-40°F. During storage the following observations and tests were made.

Color. No color difference between the four groups of cherries was noticeable.

Lesions. At periods of time indicated in Table 4, 13 boxes were removed from each group and the percent of spoiled cherries in them was estimated, on a weight basis. The results indicate that irradiation decreased the spoilage rate very slightly and only in the case of the over-wrapped fruit.

Table 4. Effect of gamma irradiation (150 krad) and subsequent storage at 39°F on the spoilage of sweet cherries. Numbers indicate sound fruit on % weight basis (sample size, 13 pints).

Overwrapped			Non-overwrapped		
Days After Irradiation	Control	Irrad.	Days After Irradiation	Control	Irrad.
1	100.0	100.0	1	100.0	100.0
3	93.5	95.7	4	92.6	95.4
6	93.8	89.9	6	87.7	91.2
8	88.6	90.1	8	87.7	91.2
10	87.6	83.2	11	81.5	85.9
13	80.5	84.7	14	67.2	73.9
16	80.7	77.7	18	60.4	67.0
20	73.6	70.6	21	56.4	56.4
24	59.6	56.5	25	47.4	47.0
31	33.2	32.4	28	47.9	51.2
38	31.0	26.8	32	33.9	43.6
			35	30.5	34.6

Texture. The texture of the cherries was measured by means of the Lee-Kramer shear press, using a technique similar to that for strawberries. A statistically significant softening due to irradiation was found, corresponding to approximately 20% decrease of the peak press reading during the first week and 10% during the 3rd and 4th weeks.

Taste. Triangular taste testing in which 15 judges participated showed no significant effect of the irradiation on the taste of the non-overwrapped cherries. The control wrapped cherries, however, were preferred over the irradiated wrapped cherries.

No overall advantage appears to be derived by irradiating (150 krad) sweet cherries, according to this experiment. It is possible, however, that the unusually dry season resulted in low microbial contamination of the fruit and the anti-microbial effect of irradiation was not demonstrable.

PRUNES

Michigan-grown Stanley prunes were exposed to 0, 50, 100, 200 and 400 krad of gamma rays, dipped in 200°F water for 30 seconds and dried in a Proctor dehydrator, at 155°F air temperature.

FIGURE 3. EFFECT OF GAMMA RAYS ON THE DRYING RATE OF STANLEY PRUNES

FIGURE 4. EFFECT OF GAMMA IRRADIA ON THE TEXTURE OF NAVY BEANS

The rate of dehydration increased as the irradiation dose increased. Under the conditions of this experiment the time to reach 25% moisture content for prunes exposed to 200 krad of gamma rays is approximately 3/4 the time for the controls (Figure 3). Although some red pigment leached out of the irradiated (200 and 400 krad) fruit during blanching, the anthocyanin content of the dry control prunes was approximately 2/3 that of the irradiated fruit. The skin of the prunes which were exposed to 200 and 400 krad broke during blanching. No flavor difference was detected between irradiated and control dry samples by a panel of 15 tasters. When the blanching was omitted no acceleration of the drying rate by irradiation was observed and no increased anthocyanin retention.

Irradiation may prove complimentary to dehydration and freeze-drying of foods.

DRY NAVY BEANS

1. Dry navy beans were exposed to several doses of gamma rays in the range of 0 to 1.6 Megarad, subsequently soaked in 4 times their weight of distilled water for 24 hours and tested by the Allo-Kramer shear press. A sharp reduction in the firmness of the soaked beans was observed as the radiation dose increased (Figure 4). The soluble solids of the soak water increased from 1.5% for the control beans to 7.0% for the beans exposed to 1.6 Megarad (Figure 5). Upon rehydration for 24 hours, the weight gain of the nonirradiated beans was 90%, that of beans exposed to 450 krad 101%, and the moisture content 54% and 56% respectively.

2. Dry navy beans were exposed to 10, 100, 400, 600, 800, and 1,000 krad of gamma rays, soaked for 16 hours and cooked in distilled water. After 0, 0.5, 1.0, and 2.0 hours of cooking the weight of the beans was determined. Table 5 summarizes the results.

Table 5.—Effect of gamma irradiation on the water uptakes of dry beans after soaking (16 hrs) and cooking. Figures indicate % of weight increase.

Dose (krad)	0	200	400	600	800	1,000
Soaking only	92.0	96.0	100.0	102.1	84.4	104.1
Soaking + 1/2 hr cooking	97.3	124.0	125.1	118.8	116.7	114.5
Soaking + 1 hr cooking	127.1	139.6	140.7	134.4	125.1	126.1
Soaking + 2 hrs cooking	144.8	144.6	164.5	141.7	135.5	133.3

It is apparent that exposure to 400 krad of gamma rays results in maximum water uptake, in general.

3. Navy beans from the market contained 10.4% moisture. One sample was dried to practically 0% moisture by vacuum, and other samples were hydrated to 19.5%, 29.3% and 43.2% moisture by soaking 2, 15 and 60 minutes, respectively. All 5 samples were exposed to 400 krad of gamma rays, and after soaking for 16 hours the water uptake and the hardness (Allo-Kramer press) of the beans was determined. The results are summarized in Table 6.

FIGURE 5. EFFECT OF GAMMA IRRADIATION ON SOLUBLE SOLIDS OF NAVY BEANS

Table 6.—Effect of gamma rays (400 krad) and moisture content of beans on their water uptake and hardness (Allc-Kramer press peak reading) after soaking.

% Moisture	Control		400 krad	
	% wt. Gain	Hardness	% wt. Gain	Hardness
0.0	90.5	74.3	91.3	43.7
10.4	91.6	79.3	106.3	36.3
19.5	91.6	79.6	100.0	41.7
29.3	94.8	76.5	103.1	42.8
43.2	93.7	74.8	97.9	51.7

The normal moisture was associated with the highest water uptake and softness after irradiation.

4. Dried beans, var. Great Northern, were exposed to 0, 300, and 600 krad of gamma irradiation and subsequently soaked for 16 hours in either equal weight of water or four times as much water. After soaking, the excess water was removed and beans were steam-blanchd for three minutes. Eight ounces of these beans were placed in no. 300 cans, covered with pork and bean sauce and the cans were exhausted, sealed, and retorted for 15, 30, 45, 60 and 75 minutes at 240°F. After retorting, the cans were cooled with water, stored at room temperature and opened 2 days later. The following observations were made on the canned beans.

1. The irradiated samples were the softest when short periods of retorting were used, but this tendency reversed as retort time increased.
2. Equal weights of soak water and beans yielded the softest beans.
3. Drained weight increased with radiation dose at brief retorting, but the reverse held true at long retort times.
4. Equal weights of beans and soak water yielded the highest drained weights.
5. There was not much difference in color development between all treatments.
6. The viscosity of the sauce in the beans soaked with an equal weight of water increased with radiation dose after 15 min. of retorting. Longer retorting reversed the trend with the sauce from the unirradiated control being the most viscous at 75 min. retorting.
7. In the case of beans soaked with an excess of water the difference in viscosity of the sauce were less pronounced.

APPLE JUICE

Juice from Michigan-grown apples, var. Northern Spy, was exposed to gamma rays in 4-ounce bottles and subjected to the following tests.

Yeast and mold count. Table 7 summarizes the results of plating one day after irradiation.

Table 7. — Effect of irradiation on the yeast and mold count of apple juice.

<u>Dose</u>	<u>Count/ml</u>	<u>% Reduction</u>
0	1400	0
100	437	68.8
200	157	88.8
300	26	98.1
400	0.5	99.9
500	0	100.0

Color. Visual observation indicated a progressive lightening in the color of the juice with increasing radiation dose. Upon storage at 50°F the irradiated juice darkened more rapidly than the control and after one month of storage there was little difference in color between the samples.

Flavor. Preliminary tasting tests on the effect of the lower doses of irradiation on the flavor of the juice were inconclusive. When larger samples of juice were exposed to 300 krad of gamma rays and compared with heat-pasteurized and non-heated juice, after one week storage of all 3 samples at 50°F, the panel found the irradiated juice inferior to the other two types of juice.

Viscosity. Table 8 summarizes the juice viscosity measurements, as determined by the Ostwald viscosimeter.

Table 8. — Effect of gamma irradiation on the viscosity of apple juice.

<u>Dose</u>	<u>Rel. Viscosity</u>
0	2.91
100	2.82
200	2.65
300	2.34
400	2.20
500	2.18

2. Local apples of the Northern Spy and McIntosh varieties were mixed and pressed in the laboratory. The juice was depectinized, clarified and packed in sterile glass bottles. The air of a number of filled bottles was removed by means of a high vacuum pump and nitrogen, free of oxygen, replaced the air in the bottles. The sealed bottles were exposed to 0, 150, 200 and 250 krad of gamma rays.

Five days after irradiation, the juice was tasted by a 15-member untrained panel and no significant difference in taste was detected between levels of irradiation, or between air and nitrogen atmospheres.

After five days of storage at 50°F, samples of the juice were plated for mold plus yeast enumeration. The results are summarized in Table 9.

Table 9. — Mold plus yeast count (per ml.) of apple juice irradiated under air or nitrogen and stored at 50°F for five days.

Control		150 krad		200 krad		250 krad	
Air	N ₂	Air	N ₂	Air	N ₂	Air	N ₂
9650	-	4850	410	820	52	88	21

These results indicate that irradiation and storage of apple juice under nitrogen results in great reduction of the mold plus yeast count.

3. Non-clarified apple juice was exposed to 0, 100, 200, 300, 400 and 500 krad of gamma rays and subsequently stored at 50°F. The periodic measurements of the viscosity of the juice are summarized in Table 10.

Table 10. — Effect of gamma irradiation and storage at 50°F on the viscosity of non-clarified apple juice. The figures are minutes of flow through an Ostwald viscosimeter.

Sample	0 wk.	1 wk.	2 wks.	4 wks.
0 krad	3.429	2.697	2.725	1.700
100 krad	3.200	2.968	2.412	2.046
200 krad	2.951	2.697	2.397	2.046
300 krad	2.615	2.430	2.249	2.161
400 krad	2.514	2.452	2.219	2.103
500 krad	2.343	2.976	2.160	2.233

These results indicate that gamma rays decrease the viscosity of apple juice immediately after irradiation. Upon storage, however, the decrease in viscosity of nonirradiated juice is more rapid than that of irradiated juice; this is probably due to pectolytic activity of microorganisms since a clarified, nonirradiated sample showed very little change of viscosity during similar storage.

4. Clarified apple juice was irradiated and stored as in (3). Part of the juice was packed under air and part under nitrogen. Periodically, the color of the juice was measured by diluting it with 3 volumes of water and determining the absorbancy of the dilution at 440 m in an Evelyn colorimeter. The results are summarized in Table 11.

Table 11. — Effect of gamma irradiation and storage at 50°F on the color of air and nitrogen packed, clarified apple juice. The values are A₄₄₀.

Sample	0 wk.		1 wk.	2 wks.	4 wks.	
	Air	N ₂			Air	N ₂
0 krad	.240	.230	.231	.189	.192	.182
100 krad	.181	.142	.174	.201	.161	.147
200 krad	.128	.107	.114	.123	.54	.137
300 krad	.096	.093	.111	.087	.125	.125
400 krad	.091	.086	.101	.087	.129	.124
500 krad	.090	.088	.101	.097	.119	.173

Both the air-packed and nitrogen-packed juice is bleached by irradiation, but upon storage the difference in color decreases as the irradiated juice darkens and the control juice becomes lighter in color.

5. Non-clarified juice was irradiated and stored as in (3). Periodically 1 volume of juice was mixed with 3 volumes of 95% ethanol, the mixture was filtered and the color of the filtrate was measured at 440 ml. in the Evelyn colorimeter. Table 12 summarizes the results.

Table 12.— Effect of gamma irradiation and storage at 50°F on the color of apple juice filtrate. (Non-clarified juice was used which was depectinated by ethanol just before color measurement.) The values are A_{440} .

Sample	0 wk.	1 wk.	2 wks.	4 wks.
0 krad	.335	.248	.233	.096
100 krad	.277	.222	.204	.114
200 krad	.229	.206	.164	.102
300 krad	.177	.150	.123	.087
400 krad	.143	.183	.118	.095
500 krad	.133	.195	.141	.079

Generally, irradiation and storage, independently resulted in bleaching of the ethanolic filtrate. The juice, however, did not lighten to the same extent visual observation indicated that the juice was much darker than the filtrate and the sediment obtained by mixing with ethanol was very dark.

SUGAR BEETS

Sugar beets were exposed to gamma rays in the dose range of 0 to 400 krad. They were then shredded and extracted with boiling water. No significant difference was found in the total soluble solids extracted, nor in the optical rotation of the extracts.

It was observed, however, that the shredded beats exposed to 50 and 100 krad irradiation darkened very little in comparison to the nonirradiated ones or those irradiated with higher doses.

POTATOES

1. Potatoes of the Arenac and Ontario varieties were exposed to 0, 2.5, 5.0, 7.5, and 10 krad of gamma rays and subsequently stored at 70°F, 45% RH for conditioning prior to making potato chips. The potatoes were evaluated periodically for weight loss, sprout inhibition, physical appearance, reducing sugar content and chipping quality.

a) Wt. loss. The results on weight loss appear in Tables 13 and 14.

b) Sprouting. Two days after irradiation sprouting could be observed in the 0, 2.5, and 5.0 krad treatments. Potatoes exposed to higher levels of irradiation displayed swelling of the buds

ut this was not developed to sprouting. The weight of the sprouts after 55 days of storage at 55°F, 45% RH is given in Table 15.

Table 13. — Weight loss of irradiated Arenac potatoes, expresses as % of original weight.

Days of Storage	Control	2.5K	5.0K	7.5K	10.0K
1	99.6	99.4	99.6	99.7	100.0
2	99.1	98.8	98.8	99.9	99.4
3	98.8	98.4	98.4	98.5	98.9
6	97.6	97.0	97.1	97.4	97.6
9	96.6	95.8	95.9	96.6	96.6
13	95.1	94.0	94.4	95.2	95.2
16	93.9	92.6	93.4	94.5	94.2
21	92.1	90.5	91.9	93.1	92.8
27	88.4	96.3	88.7	90.8	90.3
34	85.3	82.7	86.2	89.0	88.2
41	82.0	78.7	83.3	87.0	86.2
55	75.2	71.1	78.2	83.7	82.5

Table 14. — Weight loss of irradiated Ontario potatoes, expressed as % of original weight.

Days of Storage	Control	2.5K	5.0K	7.5K	10.0K
1	99.7	99.7	99.5	99.7	99.6
2	99.2	99.1	98.4	98.7	98.7
3	98.6	98.8	98.3	98.1	98.2
6	97.3	97.2	96.7	96.5	96.7
9	95.8	95.8	95.3	95.0	95.5
13	94.3	93.5	93.7	93.4	93.9
16	93.3	92.6	92.4	92.3	92.5
21	91.5	90.7	90.9	90.4	91.2
27	88.3	87.2	87.7	87.8	88.8
38	85.7	84.1	84.7	85.5	86.5
41	83.0	81.0	81.7	83.2	84.5
55	77.0	74.8	75.8	79.5	80.9

It appears that no significant differences in weight loss between control and irradiated occurred under the conditions of this experiment.

Table 15. — Sprout weight in % of total potato weight after 55 days of storage.

Variety	Control	2.5 krad	5.0 krad	7.5 krad	10.0 krad
Arenac	7.7	6.4	0.8	0.0	0.0
Ontario	5.4	4.9	2.7	0.0	0.0

2. Potato chips were made from the conditioned potatoes which had been treated as described previously. The following standard conditions were used. One half pound of sliced potatoes were fried for 3 minutes at 375°F. After draining the potatoes were graded according to the "Proposed Color Reference Standard" presented at the Production and Technical Division Meeting, National Potato Chip Institute, 1954. The reducing sugar content of the potatoes was also determined. Tables 16 and 17 summarize the results.

Table 16. — Reducing sugar content of Arenac potatoes and color of chips at various irradiation doses and post-irradiation storage periods (70°F).

Post-Irradiation Storage, Days	Control		2.5 krad		5.0 krad		7.5 krad		10.0 krad	
	%	Chip	%	Chip	%	Chip	%	Chip	%	Chip
	Sugar	Color	Sugar	Color	Sugar	Color	Sugar	Color	Sugar	Color
2	1.51	710	1.61	710	1.75	710	1.60	710	1.55	710
8	1.28	9.5	1.27	710	1.46	710	1.37	710	1.48	710
15	.97	9.0	.71	7.5	.85	8.5	.78	9.5	1.03	710
23	.67	8.5	.87	8.5	.77	8.5	.79	9.0	.83	710
30	.65	8.0	.66	7.0	.59	9.0	.68	8.0	.90	9
37	.54	6.0	.49	7.0	.98	9.0	.40	7.0	.39	8
44	.54	8.0	.27	6.0	.34	7.0	.27	6.0	.47	8
58	.59	5.0	.55	6.0	.61	7.0	.79	9.0	.80	8

Table 17. — Reducing sugar content of Ontario potatoes and chip color at various irradiation doses and post-irradiation storage periods (70°F).

Post-Irradiation Storage, Days	Control		2.5 krad		5.0 krad		7.5 krad		10.0 krad	
	%	Chip	%	Chip	%	Chip	%	Chip	%	Chip
	Sugar	Color	Sugar	Color	Sugar	Color	Sugar	Color	Sugar	Color
2	.52	8.5	.59	9.0	.58	8.0	.56	8.0	.60	8.5
8	.53	7.0	.36	7.0	.47	8.5	.63	8.0	.53	8.5
15	.30	6.5	.33	6.5	.32	6.5	.45	8.0	.50	8.0
23	.35	6.0	.37	6.5	.30	6.5	.35	5.5	.38	6.0
30	.39	6.0	.22	5.0	.30	7.0	.34	7.0	.22	5.0
37	.37	6.0	.30	6.0	.22	5.0	.24	5.0	.33	7.0
44	.18	5.0	.20	6.0	.21	7.0	.18	4.0	.24	6.0

It appears that irradiation, especially at the 10 krad dose, delays the conditioning of potatoes in terms of potato chip color. There is no clear relationship between reducing sugar content and chip color in the irradiated samples.

ASPERGILLUS FLAVUS

1. Two-week old spores of Aspergillus flavus were suspended in demineralized water and exposed to 0, 50, 100, 150, 200, 250 and 300 krad of gamma rays. The percent survival of spores, as measured by plate counting, is presented in Figure 6. No data are given for doses higher than 150 krad because the counts were too few to be significant. An approximate D value of 45 krad can be calculated indicating that A. flavus spores are rather sensitive to irradiation.

2. Two-week old spores of A. flavus were suspended in 0.075 M citrate buffers, pH 3, 4, 5, 6 and 7 and exposed to gamma rays in the dose range of 0 to 200 krad. The survival results are presented in Figure 7. It is apparent that pH has no appreciable effect on the sensitivity of the spores to irradiation.

3. Two-week old spores of A. flavus were suspended in demineralized water containing 0.01% Triton X-100 and irradiated with 1 Mev electrons, in Petri dishes. The lids of the dishes

FIGURE 6. EFFECT OF GAMMA RAYS ON
ASPERGILLUS FLAVUS IRRADIATED IN
DEMINERALIZED WATER

FIGURE 7. EFFECT OF GAMMA RAYS ON ASPERGILLUS FLAVUS SPORES, IRRADIATED IN .075M CITRATE BUFFER OF VARIOUS pH

were removed just before irradiation and replaced immediately after it; control dishes indicated that the contamination ensured using this technique was negligible. The results of the irradiation on the viability of the spores, as determined by plate counting, are summarized in Figure 8. In this figure, the results obtained in a parallel experiment in which gamma irradiation was used are also indicated. No significant difference in the effect of the two types of irradiation on the viability of the A. flavus spores is apparent.

4. Spores of A. flavus were suspended in glucose solutions to give a final concentration of 0, 10, 20, 30 and 40% w/v of the sugar. The spore suspensions were exposed to 1 Mev electrons at the dose levels of 0, 50, 100 and 200 krad.

The results are illustrated in Figure 9, and indicate that dextrose in solution increases the resistance of A. flavus spores to irradiation.

5. Two-week old spores of A. flavus were suspended in demineralized water containing 0.01% Triton X-100. One ml. of this suspension was transferred to each of 14 sterile Petri dishes which were then dried under 27" of vacuum for 4 hours at 25°C and placed in a desiccator overnight. The following day these dishes, along with another 14 dishes which contained 1 ml. of spore suspension plus 3 ml. of water, were exposed to 0, 50, 100, 150, 200, 250 and 300 krad of 1 Mev electrons. The irradiation was performed as described in (3). The results of the irradiation on the ability of the spores to form colonies are summarized in Figure 10. It is apparent that the dried spores of A. flavus are considerably more resistant than the spores which were in water suspension.

6. Two-week old spores of A. flavus were suspended in 0, 10, 20, 30 and 40% solutions of glucose, w/v. The concentration of the spores was 6×10^6 per ml., approximately. The suspensions were exposed to gamma rays from Co-60 in the dose range of 0-200 krad. The effect of the irradiation on the ability of the spores to form colonies is illustrated in Figure 11. It appears that glucose in solution decreases the radiosensitivity of the spores only slightly. The protective effect of glucose against damage by 1 Mev electrons was somewhat greater.

From the same harvest of A. flavus spores, water suspension and dried spores were exposed to gamma rays in the range of 0-300 krad. The results are summarized in Figure 12. The dry spores were more resistant to radiation damage than those suspended in water. It appears, however, that the increase in radioresistance was not so high as that observed when 1 Mev electrons were used. Since the dry spores used in the gamma and electron irradiations were not portions of the same spore preparation the difference might not be entirely due to the nature of irradiation.

FIGURE 8. EFFECT OF IRRADIATION ON ASPERGILLUS FLAVUS SPORES IRRADIATED IN DEMINERALIZED WATER

FIGURE 9. EFFECT OF 1 MEV ELECTRONS ON THE SPORES OF ASPERGILLUS FLAVUS IRRADIATED IN DEXTROSE SOLUTIONS

FIGURE 10. EFFECT OF 1 MEV ELECTRON IRRADIATION ON DRY AND WET SPORES OF ASPERGILLUS FLAVUS

FIGURE 11. EFFECT OF GAMMA IRRADIATION ON THE SPORES OF *ASPERGILLUS FLAVUS* IRRADIATED IN VARIOUS DEXTROSE SOLUTIONS

FIGURE 12: EFFECT OF GAMMA IRRADIATION ON DRY AND WET SPORES OF ASPERGILLUS FLAVUS

RADIATION-INDUCED CALCIUM RELEASE FROM PLANT TISSUES

Softening of fruits and vegetables upon exposure to ionizing radiations has been repeatedly reported. This softening has been associated with changes in the pectic substances of the irradiated tissues. The association has been clear, however, only for high (over 500 kilorad) doses of irradiation. The effect of lower doses of irradiation on the texture of plant tissues, although strong, has not been interpreted in well defined chemical changes. Since lower doses (200 to 300 kilorad) are likely to be used in the radiation-pasteurization of fruits and vegetables, it is important to understand the mechanism of the radiation-induced softening. Such understanding may facilitate the search for means to avoid the softening in the cases in which it is undesirable, or enhance it when desirable.

The premise on which the following experiments were undertaken has been that calcium significantly contributes to the firmness of fruits and vegetables, specifically through the Ca-pectin association. Should calcium be released by irradiation the texture of the plant tissue would be expected to weaken.

In a preliminary experiment, dried tomato leaves containing ^{45}Ca were pulverized, extracted with 70% ethanol and the marc was divided into two equal-weight portions. One portion was suspended in water and exposed to 595 kilorad of gamma rays from Cobalt-60, while the other portion was also suspended in water but not irradiated. After the solid phase of each suspension was separated by filtration, an aliquot of the filtrate from the irradiated marc was found to display 4,146 counts per min. (cpm) of radioactivity, but an equal aliquot of the control filtrate showed only 633 cpm.

In a second experiment, a semi-ripe strawberry fruit was severed from the plant and the stem of the berry was immersed in a solution of $^{45}\text{CaCl}_2$ (approx. 5 μc). Two days later the berry was crushed in boiling 95% ethanol, disintegrated in a Waring blender, filtered and the marc was washed with 70% ethanol until very little radioactivity could be detected in the washings. The marc was then divided into two equal portions and each portion was suspended in 5 ml. of water. One of the suspensions was exposed to 200 kilorad of gamma irradiation in a glass ampoule and the other was kept as a control; they were then filtered and the marc was again washed with 200 ml. of 70% ethanol. Filtrate and washings were mixed and the solvent was evaporated; the dry residue was dissolved in 4 ml. of 1N HCl and 0.5 ml. of the solution was transferred into a planchet, dried and measured for radioactivity by a gas-flow, thin window detector, connected with an automatic sample changer, scaler and printing timer. The radioactivity of the irradiated sample was 1,646 cpm, that of the control was 593 cpm.

In a third experiment, ^{45}Ca -labelled strawberry marc was prepared by the same method as in experiment No. 2. The suspension of the marc was irradiated in an uncovered Petri dish

with 200 kilorad of 1 Mev electrons, rather than gamma rays. The radioactivity of the non-irradiated control was 350 cpm, that of the irradiated sample 908 cpm.

The higher radioactivity of the extracts of the irradiated plant tissues can not be due to induced radioactivity, because no such induction is expected from the radiation energy levels used here. It must be due to radiation-induced release of bound calcium from the tissues. In order to show that pectin-bound calcium can be released by ionizing radiations the following experiment was conducted.

Calcium-45 pectate was prepared by precipitation of pectic acid from an aqueous solution with $^{45}\text{CaCl}_2$. The precipitate was washed with water until the radioactivity of the washings reached a practically constant value. The marc was dried in a desiccator and portions equal to 60 mg. each were suspended in 3 ml. of water. One suspension was exposed to 200 kilorad of gamma rays, a second one to 200 kilorad of 1 Mev electrons and a third suspension was not irradiated. The control and irradiated suspensions were filtered, the solid phase was washed with 100 ml. of water and the filtrate plus washings were prepared for radioactivity measurements by the procedure used in the strawberry experiments. The relative radioactivities were 73 cpm for the control, 114 cpm for the sample exposed to gamma rays and 143 cpm for the sample exposed to 1 Mev electrons.

These results indicate that calcium can be released by irradiation of plant tissues and this release may be associated with the radiation-induced softening of fruits and vegetables.

Section 3
IRRADIATION OF FOODS

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DISCUSSION

When the present research project was initiated, two cobalt-60, gamma radiation sources were available. These were described in the previous annual report for this project (1). It soon became apparent that the quantities of fish, fruits and vegetables to be irradiated for research proposed on this project were more than could be properly accommodated. The cobalt-60 was old and the irradiation zones were not designed to maintain food at refrigeration temperature during irradiation. With these facts in mind, the AEC authorized a supplement to the present contract that permitted both of these deficiencies to be corrected.

New cobalt-60 was installed in both of the UM sources, and the irradiation room at the Phoenix Radiation Facility (PRF) was air conditioned. Similar refrigeration and air conditioning equipment is presently being installed in the Phoenix Memorial Laboratory Source (PML). The strengthened PRF and PML sources now contain 4700 and 10,900 curies of cobalt-60 respectively. They provide maximum gamma ray dose rates of approximately 0.2 megarad per hour at the PRF and 1.0 megarad per hour at the PML. Lower dose rates than the maxima can be conveniently obtained at both sources by conducting irradiations outside of and at calibrated distances from the sources.

The air conditioning equipment is designed to maintain the air spaces in the irradiation chambers at 37°F. This should permit most products to be irradiated without icing; if ice is used, it should last for the length of most irradiation periods required by the research problems under way or contemplated for this contract.

It is expected that the air conditioning work now in progress at the PML will be finished early this summer. This should complete the installation and provide excellent irradiation equipment for most of the research work in progress.

REFERENCE

- 1) Irradiation Preservation of Fresh-Water Fish and Inland Fruits and Vegetables. Report No. COO 1283-12; Isotopes-Industrial Technology, UC23; TID 4500 (19th Ed.).

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