Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.fw001

Radiation Preservation of Foods

A symposium co-sponsored by the Division of Agricultural and Food Chemistry and the Division of Nuclear Chemistry and Technology at the 150th Meeting of the American Chemical Society, Atlantic City, N.J., Sept. 16-17, 1965.

Edward S. Josephson and J. Harry Frankfort
Symposium Chairmen

ADVANCES IN CHEMISTRY SERIES **65**

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AMERICAN CHEMICAL SOCIETY

WASHINGTON, D.C. 1967

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.fw001

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Library of Congress Catalog Card 67–25568

PRINTED IN THE UNITED STATES OF AMERICA

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FOREWORD

ADVANCES IN CHEMISTRY SERIES was founded in 1949 by the American Chemical Society as an outlet for symposia and collections of data in special areas of topical interest that could not be accommodated in the Society's journals. It provides a medium for symposia that would otherwise be fragmented, their papers distributed among several journals or not published at all. Papers are refereed critically according to ACS editorial standards and receive the careful attention and processing characteristic of ACS publications. Papers published in ADVANCES IN CHEMISTRY SERIES are original contributions not published elsewhere in whole or major part and include reports of research as well as reviews since symposia may embrace both types of presentation.

PREFACE

In a world where more than half the human race is ill fed and more than one-third of all food grown or raised is spoiled, ionizing energy can contribute much toward helping close the world's nutritional gap, even in the face of a rapidly increasing population. Since the turn of the century, ionizing energy has been considered a revolutionary method for preserving man's food. It was not until after World War II that the availability of radioactive isotopes and efficient machine sources provided the necessary impetus to study this new method for preserving food.

Comprehensive research programs sponsored by the U. S. Atomic Energy Commission and the Army in this country have been under way for the past 14 years. These studies were concerned with ascertaining the physical and chemical changes in foods preserved by ionizing energy, with particular emphasis upon wholesomeness, nutritional adequacy, acceptability, and absence of induced radioactivity in foods intended for consumption by humans.

A decade and a half of unprecedented intense research such as never has been applied to any other process for preserving food is now beginning to bear fruit. A broad spectrum of highly acceptable, nutritious, wholesome foods can be prepared in the laboratory as a result of the wealth of information derived from the fundamental research on physical and chemical changes produced in foods preserved by ionizing energy.

The timing of this symposium was indeed appropriate. The entire national food irradiation program had reached the transition point where research findings from the laboratory were ready for practical application, leading to production on a commercial scale. Several foods processed by irradiation are now permitted for unrestricted human consumption. Regulations have been issued by the U. S. Food and Drug Administration permitting the use of ionizing radiation to preserve bacon, to inhibit potato sprouts, and to disinfest wheat and wheat flour. Action is now pending on petitions for regulations for ham, strawberries, oranges, six species of fish, and for several polymeric packaging materials. Petitions for beef, pork, chicken, and shrimp will be submitted during the coming year. To encourage commercialization of this new preservation process, the U. S. Atomic Energy Commission is providing leadership toward private industry's constructing and operating a pilot plant for preserving meat and poultry. The Department of Defense has agreed to purchase a large portion of the pilot plant's output.

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For these reasons it was fitting that the experts in the several scientific and engineering disciplines involved in radiation preservation of foods gather together and report on the latest advances. A total of 16 formal papers were presented, covering the major aspects of chemistry and design technology associated with radiation preservation of foods.

In addition to the formal presentations, the participants and the audience engaged in vigorous discussions which continued well after the three sessions were officially closed.

It is a pleasure to acknowledge the excellent assistance received from Albert S. Henick, Eugen Wierbicki, and Martha D. Driscoll of the U. S. Army Natick Laboratories and Katherine Black of the Vitro Engineering Co., whose efforts were vital to the sucess of the symposium.

Natick, Mass.
New York, N. Y.
December 1966

EDWARD S. JOSEPHSON
J. HARRY FRANKFORT

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.pr001

Some Radiation-Induced Changes in Fresh Fruits and Vegetables

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch001

The effects of radiation upon fresh fruits and vegetables include not only the immediate and direct influence upon chemical constitution, but also the subsequent indirect consequences of physiological and biochemical alteration. The most obvious detrimental response to irradiation is softening associated with degradation of pectin and cellulose. Some radiation-induced changes in physiology associated with storage longevity have been studied. Ethylene synthesis in apples is altered by radiation. Kilorad treatment of fruit and vegetable tissue causes stimulation of respiration followed by return to near-normal rates. Investigations into the biochemical causes for this in carrots indicate little alteration of either pentose-phosphate or Embden-Meyerhof glucose metabolism. Although little effect on pyruvate decarboxylation was found, there was a marked variation in acetate metabolism.

The radiation preservation of fresh fruits and vegetables has received considerable attention as one of the promising applications for food irradiation. As with most other applied aspects of food irradiation, however, the process is not without complications. This paper is concerned only with the effect of gamma radiation upon fresh commodities, drawn principally from work conducted at this laboratory. No effort is made to cover all of the changes occurring but rather only a few which illustrate the problems or limit the practical application of the process.

First, it must be recognized that fresh fruits and vegetables, unlike meat, fish, or dairy products, are living tissues. As long as they remain living and in a favorable state of health, they usually remain in a condition which is considered edible. Hence a consideration of the effects of ionizing radiations on fresh fruits and vegetables is principally a consideration of the effect upon life processes.

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Any factor which slows down the life processes but does not stop or modify the metabolism to any great extent tends to extend the storage life of that tissue. However, further complications may influence the storage of these commodities. One is spoilage through microorganism contamination. Fresh fruits and vegetables may become infected by various bacterial or fungal organisms either before or following harvest, which result in spoilage. Furthermore, we eat fresh fruits and vegetables which are appealing to the eye. We do not, for example, care to eat apples with the skin blackened with storage scald, even though the flavor, nutritive qualities, and texture have been otherwise unaltered by the physiological disorder.

The resistance of fruits and vegetables to ionizing radiations relative to that of mammals and many insects is impressive. One can deliver many thousands of rads of gamma radiation to many commodities without any significant effect. On the other hand, there is a distinct upper limit to the dose that may be delivered without bringing about immediate undesirable changes, and this, unfortunately, is below the sterilizing dose for many pathogenic molds and bacteria.

To circumvent this difficulty, at present we must be content to use pasteurizing doses of radiation. Brown-rot control on peaches (*3*) and mold control on strawberries (*20*) and citrus (*3*) have been prime areas of investigation. An interesting phase of investigation growing out of pasteurizing-dose research is the extension of shelf life owing to modification of normal life processes. For example, low doses of radiation prevent sprouting in potatoes (*7*, *27*) and onions (*8*), delay ripening in certain fruits (*19*, *25*, *29*), or retard the development of storage scald in apples (*16*, *21*). Although we are just beginning to understand the biochemical and physiological changes behind these responses, investigations in this area are resulting in many important developments.

Texture Changes

Undoubtedly the most serious detrimental response of fresh tissues to sterilizing doses of radiation is softening. It has been noted repeatedly that tissues become progressively less resistant to compression, or "mealy" in texture, with increasing doses of radiation over a certain threshold. This has been associated with degradative changes in the middle lamella of plant cells, resulting in a lower resistance of the tissues to shear and compression forces (*6*, *11*, *22*). Studies on the chemistry of this softening have indicated that these changes are principally related to radiation-induced degradation in the structural polysaccharides (*13*, *15*).

We have studied the effect of irradiation on both pectin and cellulose degradation extensively, both from the standpoint of the in vivo degradation as measured by the characteristics of the extracted polysaccharides (*13*, *16*) and in model systems utilizing in vitro techniques (*10*, *12*, *14*, *26*,

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30). As a result of these studies it was concluded that both pectin and cellulose were degraded by approximately the same dose at which tissue softening could be first demonstrated and progressed with increasing dose in a manner similar to that of softening itself (Figure 1). The degradation of pectin has been characterized by the loss of viscosity in the individual fractions, loss of specific viscosity calculated for a constant uronide or calcium pectate value, and increase in the soluble-insoluble pectin ratio. Cellulose degradation was characterized by a decrease in the specific viscosity calculated for a standard calculation.

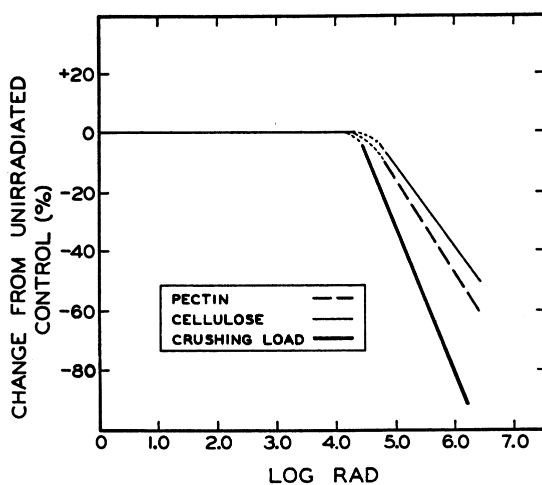

Figure 1. Over-all relation between softening of irradiated apple tissues as measured by crushing load and degradation of tissue pectins and cellulose as measured by changes in viscosity (13)

The effect of gamma radiation on the structure of pectin was studied further by subjecting semipurified citrus pectin to electrophoretic analysis following various doses of irradiation (*26*). The results of this study showed that whereas no extra components are produced upon irradiation, random fissure of the glycosidic linkages is created along the length of the pectin molecule. The increase in heterogeneity above doses of 200 krads indicate that the fragments have slightly wider distribution in charge-to-mass ratio according to the location of the fissure in relation to the free carboxyl groups. The hydrolytic fissures result in a lower weight–average molecular weight. Further analysis of the data indicates the absence of changes such as de-esterification which would alter the charge on the pectin molecule.

The degradation of pectin irradiated under various conditions of moisture has also been studied (*30*). Changes in intrinsic viscosity of dilute pectin solutions were used as a measure of the degree of degradation. Pec-

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tin was found to be least susceptible to degradation in the range of approximately 10–95% moisture. Below 10% moisture, degradation decreases sharply with increasing moisture content, while above 95% the opposite is true. Thus, we have a pronounced protective effect of water at the low moisture levels and a sensitizing effect at the higher moisture levels. Further analysis of the data indicates that the principal effect is directly on the pectin molecule at the low moisture levels, indirectly on the aqueous medium at the high moisture levels, and by a combination of both in between.

We have also found it possible to reverse this radiation-induced degradation in vitro after adjusting the pH to 1.4–2.6 with inorganic acids. Under these conditions, relatively low doses of radiation induce the formation of gels through cross-linking in both pectin (*32*) and several other polysaccharides, including carboxymethylcellulose (*31*). Gel strength depends on pH, concentration of the polysaccharide, temperature, radiation dose, and dose rate. The presence of many materials such as sugars, oxygen, organic acids, and many oxidizing and reducing agents interferes with this synthetic reaction. We are endeavoring to induce these constructive changes in vivo, but so far have met only limited success.

In summary, although we have learned much about the chemistry of softening in plant tissues, we are still studying the mechanism and mode of action of this process in order to evaluate the possibility of its control. Hence, tissue softening at even pasteurizing dosages still continues to be a major factor limiting radiation processing of fresh fruits and vegetables.

Physiological Changes

The storage life of some fresh fruits and vegetables may be extended by modifying the physiological processes with relatively low doses of radiation. Examples include delayed ripening in pears (*25*), tomatoes (*29*), and bananas (*19*), and the control of storage scald and brown-core in apples (*16*, *21*). Large variation in this response owing to species and variety can exist. In addition, the physiological state of the tissues at the time of treatment owing to such factors as maturity or environment may be of extreme importance.

Some varieties of apples which were treated with kilorad doses of radiation did not mature as rapidly as the unirradiated control during subsequent storage (*16*). This was most obvious for texture but significant with other indexes of maturity as well. This suggests that radiation may be acting through indirect means by influencing some tissue component which is itself responsible for ripening, such as ethylene. This endogenous chemical is known to exert a profound influence upon the ripening rate of many fruits. Although it is an open question whether endogenous ethylene is the cause or result of accelerated respiratory activity, its production in ir-

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radiated lemon (*18*) and preclimacteric avocado (*33*) fruits has been demonstrated.

The effect of radiation upon ethylene content of apples was investigated. The fruit were harvested at commercial maturity and either used immediately or stored for short periods under commercial-type refrigerated conditions until used. Irradiation was conducted at room temperature, usually over an 18-hour period. Ethylene samples were obtained directly from the core cavity through an 18-gage hypodermic needle inserted through the flesh of the fruit and sealed with a serum bottle stopper. Samples of internal atmosphere were collected by inserting a smaller hypodermic needle through the larger one and withdrawing a 0.2-cc. sample with a gas microsyringe. These samples were then injected directly into a modified Research Specialty dual-flame gas chromatograph. As modified for this procedure, the chromatograph used a 6 foot × 2 mm. column packed with 20% diethylene glycol succinate on Anakron A maintained at 25°C. and flushed with nitrogen as a carrier at a flow of 20 cc. per minute. Under these conditions, ethylene had a retention time of about 2 minutes.

The effect of various doses of radiation upon the internal ethylene concentration of climacteric McIntosh apples for the 10-day postirradiation period is indicated in Figure 2. Increases in ethylene concentration at doses of 5 and 10 krads were obtained immediately. At 50 and 100 krads there was an initial reduction in concentration and a subsequent rise, followed by a rather sharp decline. Experiments conducted by irradiating the fruit under anaerobic conditions indicate that whereas some of the destruction may be oxidative in nature, a considerable amount may take place under reducing conditions.

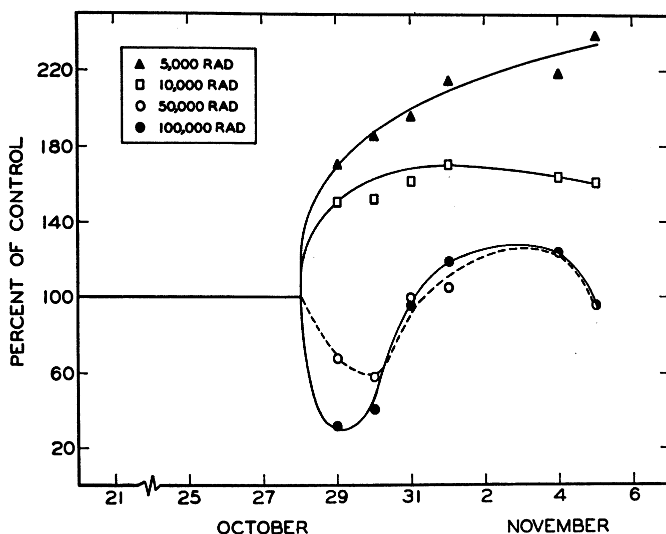

Figure 2. Effect of radiation dose on internal ethylene concentration in McIntosh apples

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The effect of various doses of radiation upon the ethylene concentration during longer periods of refrigerated storage was studied with Rome Beauty apples (Table I). The post-climacteric fruit were irradiated at room temperature and stored under refrigeration. The high values found for the irradiated fruit at the beginning of the experiment may be caused by the increased ethylene production stimulated by the radiation treatment (Figure 2). Ethylene concentration was markedly reduced by all radiation doses for the major portion of the storage period.

Table I. Ethylene Content of Rome Beauty Apples during Postirradiation Storage[a]

Radiation Dose, krads	Ethylene Content at Indicated Date									
	Dec. 17		*Jan. 2*		*Feb. 24*		*April 15*		*May 12*	
	p.p.b.	*%*	*p.p.b.*	*%*	*p.p.b.*	*%*	*p.p.b.*	*%*	*p.p.b.*	*%*
Control	70	100	36	100	30	100	92	100	74	100
5	—	—	43	116	23	77	69	74	78	104
10	—	—	45	123	14	64	54	59	101	134
50	—	—	95	260	19	63	67	73	42	56
100	—	—	75	207	16	54	89	97	51	69

[a] Expressed as parts per billion (p.p.b.) of internal atmosphere and as percent of unirradiated control. Fruit harvested Nov. 14, 1963, irradiated Dec. 22, 1963.

Radiation can alter the internal concentration of ethylene in both climacteric and postclimacteric apples, the direction of alteration depending on dose and time. Both an increase and a decrease in concentration in the internal atmosphere can be demonstrated. Some of the destructive effect is of an oxidative nature, and some is not. It is possible that these observations are related to the rate of ripening in irradiated fruit.

Changes in Respiration and Intermediary Metabolism

The effect of radiation on the biochemical processes of fresh fruits and vegetables is of considerable interest from the standpoint of its influence on shelf life. A number of studies on the effect of radiation on respiration have indicated a general stimulation of both oxygen uptake and carbon dioxide evolution during the irradiation period, subsiding to near-normal rates shortly following the cessation of treatment (*21, 23, 24, 28*).

The cause and mode of action of this response are being investigated in this laboratory. For these studies carrot tissues were chosen. Mature roots of several varieties and from several sources were used. Numerous checks between lots failed to distinguish any qualitative difference in the response obtained although there were occasionally quantitative differences. The carrots were stored at 36°F. at high relative humidity but allowed to warm to ambient temperature before use.

Preliminary experimentation on whole carrots utilizing previously reported techniques (*17*) established the respiratory response of the com-

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modity to radiation as similar to those previously reported for other fruits and vegetables (Figure 3). The during-irradiation response, however, is somewhat less than with other tissues.

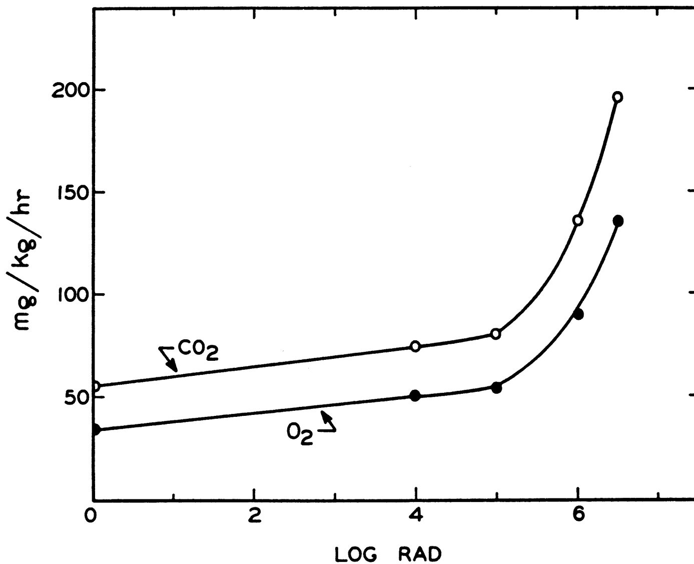

Figure 3. Oxygen consumption and carbon dioxide evolution from carrots during 18-hour irradiation

A number of studies concerned with the effect of irradiation upon intermediary metabolism in carrots have been conducted. One such study utilizing the methods of Ap Rees and Beevers (*1, 2*) involved measuring the relative radiation susceptibility of the pentose phosphate (PP) and the Embden-Meyerhof (EM) systems in the postirradiation period.

Immediately following irradiation, the carrots were sectioned, and cylinders of tissues comprising both phloem and xylem were removed with a No. 4 cork borer and cut into sections 0.5 mm. thick with a hand microtome. The disks were cut into 0.03*M* phosphate buffer at pH 5.0, rinsed twice and collected immediately on a paper towel. The sample pieces were then weighed and transferred to standard Warburg flasks. Respiratory pathway was assessed by adding either 0.025 m*M* 2,4-dinitrophenol (to increase the participation of the EM system) or 1.0 m*M* methylene blue (that of the PP system). Approximately 0.75 gram of tissue disks was used in 3 ml. of fresh buffer alone or containing 1.5 mg. of glucose, both with and without inhibitor. Readings were taken in the usual manner during incubation at 25°C.

The results of these experiments are summarized briefly in Table II. The stimulation caused by adding both metabolic inhibitions on the unirradiated control tissue is not obtained with tissues receiving increasing

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doses of radiation. Although the effect of radiation upon the PP system is approximately the same at lower doses as upon the EM system, the latter is inhibited more at the higher dose. However, there appear to be no striking differences in the susceptibility of the two systems to radiation.

Table II. Influence of Metabolic Inhibitors on Rate of Oxygen Consumption of Irradiated and Nonirradiated Carrot Disks with and without Preincubation with Glucose

Radiation Dose	*Buffer*			*2,4-Dinitrophenol*				*Methylene Blue*			
	Alone	*Glucose*		*Alone*		*Glucose*		*Alone*		*Glucose*	
krads	*Rate*	*Rate*	*%R*[a]	*Rate*	*%R*[a]	*Rate*	*%R*[a]	*Rate*	*%R*[a]	*Rate*	*%R*[a]
0	60.9	61.9	2	105	72	108	74	95	56	97	57
50	61.7	59.7	−3	106	72	122	104	91	47	94	57
100	71.8	69.1	−4	111	55	126	82	99	38	99	43
500	20.8	18.5	−11	12.5	−40	13.6	−26	25	22	16	−16

[a] % response = % stimulation of appropriate unincubated or preincubated tissue slices receiving same irradiation treatment.

Intermediary metabolism of irradiated tissues was examined to attempt to explain the increased respiration on the basis of biochemical mechanics. In these experiments carrot disks 0.5 mm. thick were prepared as before and subjected to substrate utilization studies (*9*). This involved incubating the slices with the appropriately labeled substrate in 0.03*M* phospate buffer at 25°C. The incubation involves shaking with gentle aeration and trapping the respired CO_2 in alkali. Following incubation the slices were removed, washed, ground with 80% ethanol, and filtered, and both the filtrate and residue were assayed for radioactivity. Thus, a measure of substrate absorption and utilization was determined.

Glucose metabolism was found to be only slightly affected by radiation, this effect being a small reduction in the evolution of respiratory CO_2 (*5*). In a similar manner pyruvate catabolism was found to be slightly inhibited by radiation, resulting in a reduction of CO_2 evolution from the carboxyl carbon. These results are in contrast to those obtained in the respiration experiments, in which a stimulation in CO_2 evolution was found. The EM system was slightly more sensitive to radiation than the PP system, verifying the results of the Warburg experiments above.

Further analysis of glucose and pyruvate metabolism revealed that radiation reduced the amount of substrate incorporated into insoluble cellular material. An increased absorption of substrate was also found, probably caused by the radiodestruction of the semipermeable qualities of the cell membranes and the resulting absorption by purely passive mechanisms. Supporting experiments on the direct radiodegradation of glucose have shown that the results obtained are caused by alterations in the metabolic reactions themselves and not to a radiodegradation of the glucose per se.

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These studies indicate a general reduction in all metabolic activity of the irradiated system and in accumulations of the substrates glucose and pyruvate. This cannot explain the observed increased respiratory rate of the tissue following irradiation.

Further experimentation was conducted on the effect of radiation on acetate metabolism (*4*). These experiments utilized the techniques described above. Irradiation stimulated catabolism of acetate to CO_2 with resulting increase in the evolution of radiocarbon dioxide. A proportional increase was noted for both the carbons, suggesting that the complete cycle was operating in the normal fashion. The increase in respiratory CO_2 was shown to be proportional to the dose. However, in these experiments of limited duration, a threshold of about 200 krads was necessary for a significant stimulation. A dose of 1000 krads induced a 30% increase in the CO_2 evolution. Measurement of anabolic function in irradiated tissues indicated a reduced incorporation of acetate into cellular-insoluble material. The incorporation of both carbons of acetate has been shown to decrease proportionally, suggesting that the complete acetate moiety is being incorporated. The majority of the total cellular activity was found in the ethanol extract of the tissue, and chromatographic analysis proved this to be acetate. It is believed that the effect of radiation upon acetate metabolism explains the increased CO_2 evolution detected in the respiration experiments. It is postulated that the normal energy source of the tissue stems from citric acid cycle activity and that radiation stimulated this pathway, resulting in an increased rate of respiration.

The absorption of labeled acetate, unlike that of glucose and pyruvate, was reduced with increasing radiation. At 1000 krads the absorption of acetate was only 1% that of the unirradiated control. This marked reduction is not to be expected on the basis of an increased acetate catabolism and reduced anabolism combined with the predicted increase in cell membrane permeability. One explanation of this anomaly could be the increased availability of acetate from within the cell itself, resulting in an increased acetate pool. Accordingly, the conversion of acetate into protein and lipids under the influence of radiation was studied.

Lipid synthesis from acetate was found to be sensitive to radiation. At a dose of 500 krads there was a 50% decrease in total lipid production, and at 1000 krads there was an 80% decrease. An equal reduction in the incorporation of both acetate carbons was found, indicating that the major portion of lipid was synthesized from the intact acetate moiety.

Determination of the susceptibility of individual amino acids to radiodegradation has shown that, in most instances, their degradation in tissue is proportional to the dose received. Only the glycine and leucine contents appear to be relatively radiation-resistant. The synthesis of amino acids from acetate is somewhat more sensitive to radiation inactivation, a dose of between 500 and 1000 krads completely inhibiting synthesis of all

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the amino acids except serine, glutamic acid, and aspartic acid. It is apparent that not only is more acetate available to the oxidative pool because of reduced amino acid synthesis, but free amino acid degradation products are added to the metabolic pool.

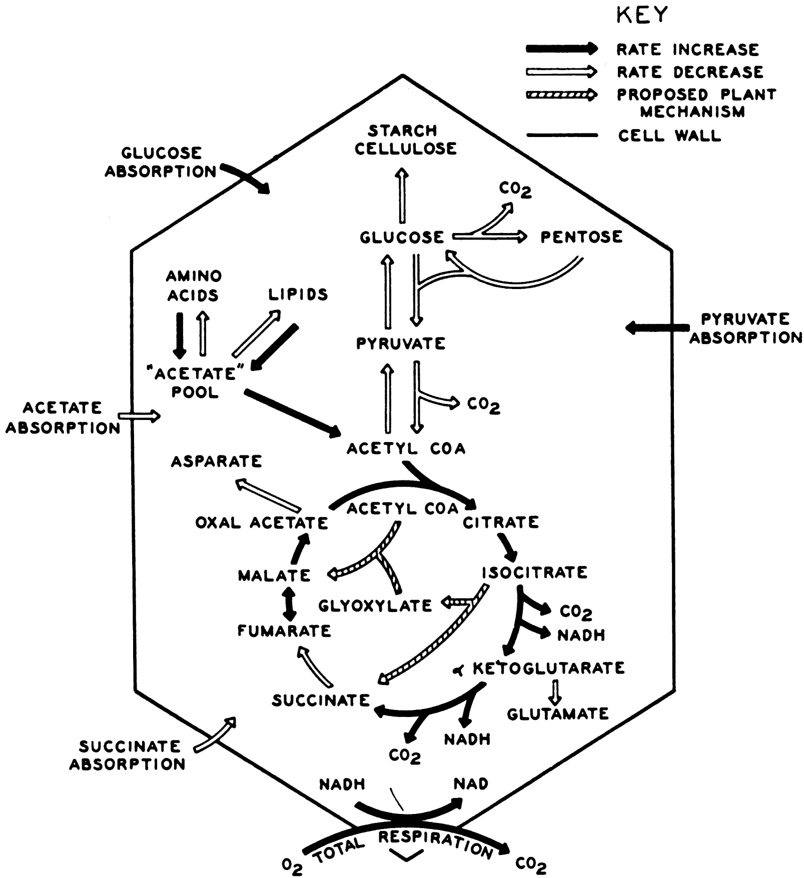

Figure 4. Influence of gamma radiation on intermediary metabolism of carrot slices (5).

Figure 4 summarizes the result of these experiments. All reactions associated with carbohydrate metabolism are decreased by exposure to radiation, while all associated with the citric acid cycle and acetate catabolism are increased. Also, in every case studied, anabolic reactions were reduced by radiation.

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RECEIVED November 9, 1965. Approved by the Director of the New York State Agricultural Experiment Station, Geneva, N. Y., as Journal Paper No. 1450, July 29, 1965. Work supported in part by a research grant (EF-00490) from the National Institutes of Health, Division of Environmental Engineering and Food Protection, and in part by a research contract (AT(30-1)-3774) from the U. S. Atomic Energy Commission, Division of Biology and Medicine [Publication AT(30-1)-3774-4].

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch001

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Irradiation Flavor and the Volatile Components of Beef

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch002

Knowledge of the volatile components of irradiated and nonirradiated beef is reviewed. Concurrent and nonconcurrent irradiation procedures produce the same compounds but in different relative quantities. Storage of irradiated beef decreases irradiation flavor and the quantity of volatile constituents. Methional, 1-nonanal, and phenylacetaldehyde are of primary importance in beef irradiation off-flavor produced under the conditions described.

A problem associated with beef sterilized by irradiation at approximately room temperature is the production of an unpleasant flavor and aroma. This paper summarizes knowledge of the volatile components of enzyme-inactivated irradiated and nonirradiated beef, reviews the effects of concurrent and nonconcurrent irradiation procedures and of storage on these components, and presents evidence that methional (3-methylmercaptopropionaldehyde), 1-nonanal, and phenylacetaldehyde are of primary importance to irradiation off-odor in beef thus processed.

Any basic study of the chemistry of irradiation flavor is complicated by the fact that volatile components of nonirradiated beef must be known. Otherwise, those components produced by irradiation (and thus may be responsible for off-flavor) and those which are normally present in nonirradiated beef cannot be determined.

Knowledge of the volatile constituents of both raw and enzyme-inactivated, irradiated, and nonirradiated beef has been accumulated through the efforts of several research groups (*1, 3, 11, 14, 17, 18*). Components of irradiated raw beef which were identified before 1962 are summarized in Table I. These compounds were isolated from ground, lean, raw beef samples which exhibited irradiation off-odor as a result of doses ranging from 2 to 6 megarads in the presence of oxygen and at roughly room temperature. Isolation methods were gas entrainment of volatiles from aqueous

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

Table I. Components of Irradiated Raw Ground Beef

	Basic and Alcoholic Constituents	
Ammonia[a]	Ethylamine	Methanol[a]
Methylamine	4 unknown amines	Ethanol[a]
	Sulfur-Containing Components	
Hydrogen sulfide[a]	3-(Methylthio)-propionaldehyde	Dimethyl disulfide[a]
Methyl mercaptan[a]	Dimethyl sulfide[a]	Diethyl disulfide
Ethyl mercaptan[a]	Carbon disulfide	Ethyl isopropyl disulfide
n-Propyl mercaptan	Methyl ethyl sulfide	Diisopropyl disulfide
Isobutyl mercaptan	Methyl isopropyl sulfide	Carbonyl sulfide
A C_5 mercaptan	Diisopropyl sulfide	
	Carbonyl Components	
Acetaldehyde[a]	Pentanal	Propanone[a]
2-Propenal	Hexanal	2-Butanone[a]
2-Butenal	Heptanal	3-Buten-2-one
	Hydrocarbons	
Ethylene	1-Heptene	*n*-Hexane
Propene	1-Octene	*n*-Heptane
1-Butene	Propane	*n*-Octane
1-Pentene	*n*-Butane	Benzene
1-Hexene	*n*-Pentane	

[a] Also present in nonirradiated beef isolates, but in smaller quantity.

slurries, vacuum distillation, or concurrent radiation-distillation. Of the basic and alcoholic constituents of raw irradiated beef, ammonia, one of the unknown amines, methanol, and ethanol were known to be components of nonirradiated raw beef. The sulfur-containing components of irradiation odor isolates include hydrogen sulfide, methyl and ethyl mercaptans, dimethyl sulfide, and dimethyl disulfide, which are also found in nonirradiated beef but in much smaller quantity. Of the carbonyl components, acetaldehyde, propanone, and 2-butanone are present in nonirradiated beef in smaller amounts. Several hydrocarbons were identified as components of irradiated raw beef. In general, the greater the radiation dose, the greater the quantity of volatile constituents produced.

The contribution to irradiation off-flavor of individual components was unknown, though the sulfur- and nitrogen-containing substances were suspected to be significant because of their inherent strong unpleasant odors. Merritt (*9*) suggested that dimethyl sulfide, 1-hexene, and *n*-hexane were important components of irradiation odor and pointed out that the quantity of these compounds produced increased directly with radiation dose.

A digression from irradiated beef components to the few known constituents of beef in the form of beef extract or beef broth is of interest. The substances listed in Table II have been identified (*2*, *7*, *19*) in various extracts of cooked ground beef. The fact that many of these components of

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch002

one of the most acceptable odors known are also found in the bland aroma of raw beef and in the unpleasant odor of irradiated beef emphasizes an important lesson now well known to flavor chemists. Thanks to modern instrumentation, complex mixtures of volatile components can be separated and their components identified in almost every foodstuff. However, many of the same components are found in a wide variety of food materials. The major challenge, therefore, is to select from the many components present those which contribute to the odor or flavor under investigation.

Investigations in our laboratories have been concerned with characterizing odor components produced by irradiating approximately 15-pound batches of ground, raw, lean beef, vacuum-packed in sardine cans and enzyme-inactivated (*4*). Irradiation at 5 megarads was carried out either in the M.I.T. cobalt-60 source before removing volatile constituents by distillation, or concurrent radiation-distillation at 5 megarads was carried out by procedures described elsewhere (*15*, *17*).

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The concurrent method allowed aqueous beef slurries to be irradiated (in the presence of oxygen) and at almost the same time volatile components were removed at pressures of about 25 mm. of Hg and at a temperature around 32°–36°C. When irradiation was carried out before distillation, the cans of irradiated beef were opened immediately or after 6-months' storage at ambient temperature. The beef was then slurried, and distillation was carried out in the usual manner (*15*, *16*). Nonirradiated beef slurries were distilled in exactly the same way as were periodic "blank" distillations of distilled water, to allow detection of contaminants or artifacts contributed by the distillation apparatus. In all cases, one condensate was collected at 0°C. (distillate) and another at −78°C. (traps).

All distillates were saturated with sodium chloride and extracted with diethyl ether. The ether extracts were dried over anhydrous sodium sulfate and then concentrated by careful distillation to the minimum practical volume (about 1 ml.). The yields of the resulting odor concentrates (on an ether-free basis) were estimated by gas chromatography.

Total yields of odor concentrates obtained from concurrently and nonconcurrently processed, freshly irradiated enzyme-inactivated beef and from stored (6 months) irradiated beef are summarized in Table III. The de-

Table II. Volatile Components of Cooked Ground Beef

Ammonia (*7*, *19*)	Propanone (*2*, *7*, *19*)
Methylamine (*7*)	2-Butanone (*2*)
Hydrogen sulfide (*2*, *7*, *19*)	Diacetyl (*19*)
Methyl mercaptan (*2*)	Formic acid (*19*)
Ethyl mercaptan (*2*)	Acetic acid (*19*)
Dimethyl sulfide (*2*, *19*)	Propanoic acid (*19*)
Formaldehyde (*7*)	2-Methylpropanoic acid (*19*)
Acetaldehyde (*2*, *7*, *19*)	Lactic acid (NH_4 salt) (*7*)
Propanal (*2*)	Methanol (*2*)
2-Methylpropanal (*2*)	Ethanol (*19*)
3-Methylbutanal (*2*)	

Table III. Summary of Yields of Odor Concentrates

Treatment	*Yield, p.p.m.*
Nonirradiated	30.8 ± 7.9
Concurrent radiation-distillation	43.3 ± 7.8
Radiation prior to distillation	36.2 ± 12.8
Irradiated and stored for 6 months	9.7 ± 0.2

tailed results on which this information is based have been published (*16*). Variations in the yields from any single treatment are believed to result from experimental difficulties encountered when measuring, on the one hand, kilograms and many liters of materials and, on the other hand, small volumes of volatile ether solutions. With the exception of stored beef, little difference between the amounts of odor concentrates isolatable from irradiated and nonirradiated beef was noted, even though all concentrates from irradiated beef strongly exhibited irradiation off-odor. Concentrates from nonirradiated beef had a normal bland meat odor. Isolates from the stored irradiated beef were also bland and not like irradiation odor.

The odor concentrates were separated by temperature-programmed gas chromatography on a 20% Carbowax 20M column. Typical chromatograms obtained from the distillate—i.e., the odor concentrate isolated

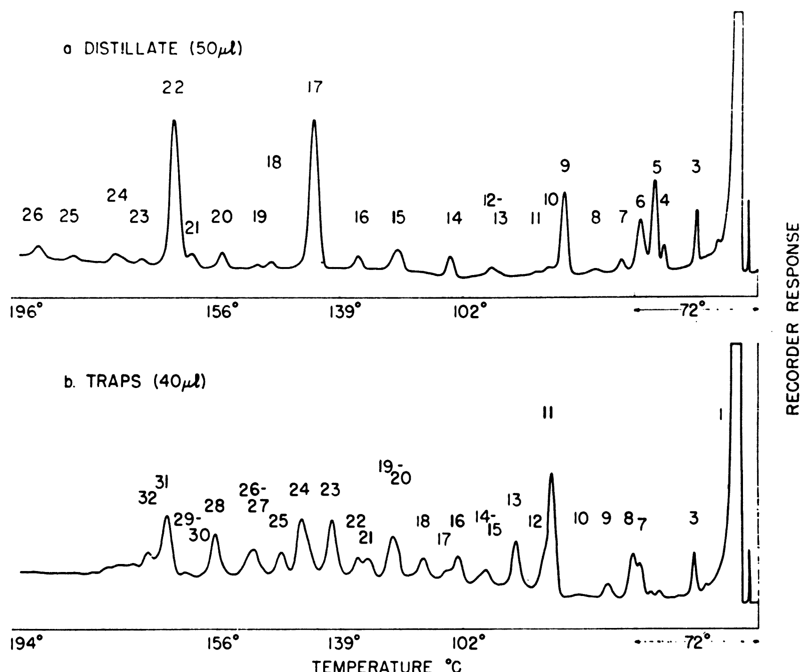

Figure 1. Temperature-programmed separation of irradiation flavor isolates on a 20% Carbowax 20M column

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch002

from the material condensed at 0°C. during distillation—and from the traps —i.e., the odor concentrate isolated from the condensate collected at −78°C.—are given in Figure 1. The difference in pattern between these chromatograms illustrates to some degree the distribution of components between the two condensate collection temperatures.

Figure 2 compares the distribution of components in a nonirradiated isolate (0°C.) with an irradiated isolate. The solid curve resulted when the beef had been irradiated at the cobalt-60 source and then distilled. The dashed curve shows separation of concurrently processed isolates. Figure 3 illustrates the decrease in volatile constituents brought about by storage.

The instrument used to obtain these chromatograms was fitted with a katharometer detector and a 6-foot 1/4-inch i.d. column. When a flame ionization detector was used, about 25 additional components, eluted after the peaks shown in Figures 1 and 2, were detected.

Preparative separations of 40- or 50-μ liter samples were obtained on a 2-meter, 4-mm. i.d., stainless steel column packed with 20% Carbowax 20M on Chromosorb P (60- to 65-mesh). The column was programmed from approximately 70° to 196°C. at the rate of 2.4° per minute.

Fractions were trapped at the detector outlet in glass U-tubes chilled in liquid nitrogen. The tubes were sealed and stored in a freezer until further investigations could be made.

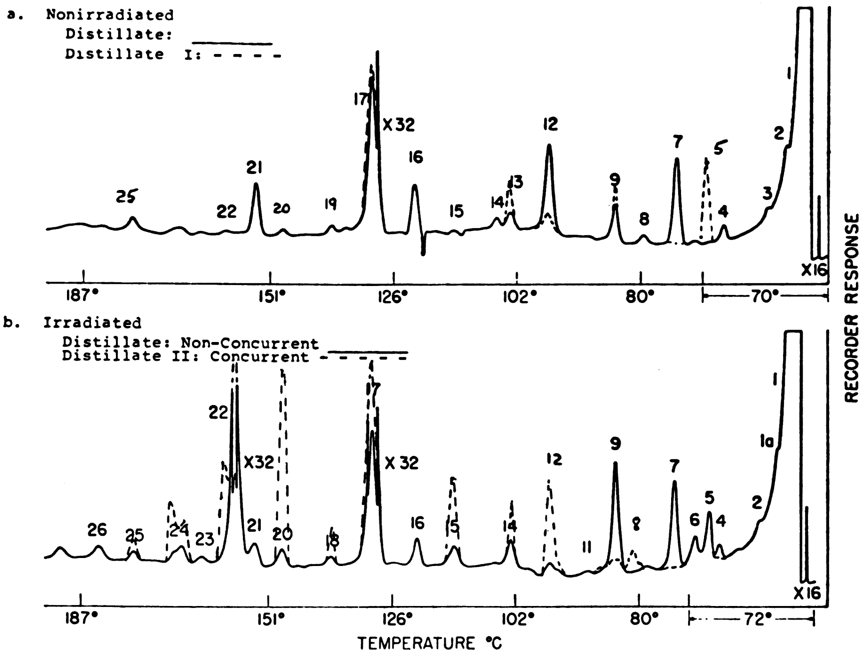

Figure 2. Temperature-programmed separation of isolates from nonirradiated and irradiated beef on a 20% Carbowax 20M column

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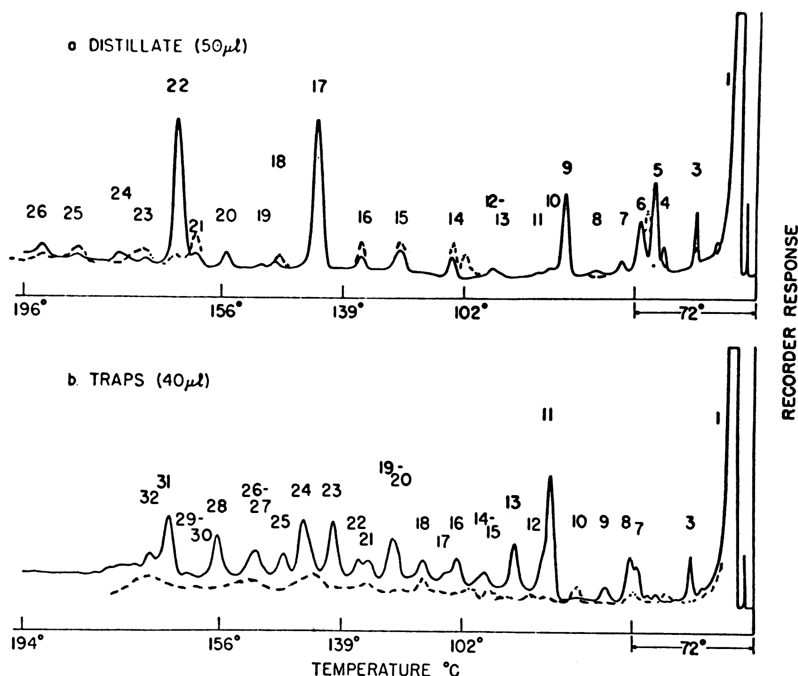

Figure 3. Temperature-programmed separation of irradiation flavor isolates on a 20% Carbowax 20M column

——— *Not stored*
— — — *Stored*

Infrared spectra of individual fractions were determined by means of a Beckman IR-5 spectrophotometer equipped with a 5 × KBr lens-type beam condenser. Infrared spectra of selected reference compounds were obtained from samples which had been purified by chromatography. On the basis of identity of infrared spectra and retention data with those of authentic reference compounds, most of the peaks shown in Figures 1 and 2 were identified (*15, 16*). To obtain information about minor components not detectable in the infrared spectra, mass spectra were obtained as components of an irradiated odor concentrate were eluted from a 10-foot, 1/8-inch 5% Carbowax 20M column programmed from 20° to 160°C. at 1° per minute. These spectra were obtained on a modified model 14 Bendix Time-of-Flight mass spectrometer. Electron energy was set at 70 e.v., and spectra were scanned from m/e 14–200 in 6 seconds.

Interpretation of the resulting spectra and comparison with known reference spectra obtained in the same manner confirmed the presence of the substances already identified and allowed us to identify additional hydrocarbons. All constituents of freshly irradiated beef identified during our investigations are summarized in Table IV.

Because of sensory evidence (described below) that no significant contribution to irradiation odor was made by substances eluted after those

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch002

Table IV. Average Composition of Total Odor Concentrates from Nonirradiated and Irradiated Beef

Compound	*Nonirradiated,* %	*Concurrent Irradiation,* %	*Nonconcurrent Irradiation,* %
Ethyl acetate	—	~.83	0.97
1-Butanal	—	~.83	—
2-Butanone	4.78	2.72	4.09
Unknown	1.85	—	—
Ethanol	3.70	~2.48	~2.54
2-Propanol	0.28	~1.49	~1.06
n-Nonane	—	—	1.48
1-Nonene	—	—	1.46
Benzene	4.85	0.32	2.25
1-Pentanal	2.09	1.17	0.92
2-Butanol	12.04	0.72	4.57
Unknown	0.24	0.15	0.24
n-Decane	—	0.20	5.42
1-Decene	—	0.62	2.22
1-Hexanal	22.86	9.80	2.12
1-Butanol	1.73	~1.98	1.33
3-Methyl-1-butanol	0.66	—	~0.69
n-Undecane	—	~0.29	2.26
1-Undecene	—	0.25	1.32
1-Heptanal	2.86	8.26	~3.31
1-Pentanol, 2 unknowns	5.60	1.80	1.08
n-Dodecane	—	—	2.44
1-Dodecene	—	1.66	4.32
Acetoin 1-Octanal	22.90	9.93	17.11
1-Hexanol	1.98	1.25	2.50
Unknown	0.58	—	—
n-Tridecane 1-Tridecene	—	0.65	3.12
1-Nonanal	2.52	26.99	3.40
1-Heptanol	5.27	4.52	1.71
Methional *n*-Tetradecane[a] 1-Tetradecene[a]	0.26	16.47	18.08
1-Decanal *n*-Pentadecane[a] 1-Pentadecene[a]	0.34	0.71	0.54
1-Octanol	0.99	2.34	~2.82
1-Undecanal	1.05	0.60	0.48
Benzaldehyde *n*-Hexadecane[a] 1-Hexadecene[a]	0.78	2.05	~2.82
Phenylacetaldehyde	0.40	0.37	1.22

[a] Identification based on mass spectra.

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shown in Figures 1 and 2 or by several fractions still remaining unknown, no further mass spectral analyses were carried out.

The quantitative composition of odor concentrates was estimated from chromatograms analogous to and including those shown in Figures 1 and 2. It was assumed that all important odor components had been eluted from the column. This was justified by the fact that when all the fractions shown on irradiated beef chromatograms were collected in a single trap, the trap exhibited irradiation off-odor. Based on this same observation, it was concluded that the components eluted after the last fractions shown in Figures 1 and 2, and detected only on chromatograms obtained with a flame ionization detector, did not contribute significantly to irradiation off-odor. Thus, only components in chromatograms like those in Figures 1 and 2 determined by thermal conductivity detection were investigated intensively.

Based on the method of internal normalization of peak areas, the percentage composition (less the diethyl ether solvent shown as the initial large component in Figures 1 and 2) of odor concentrates was estimated. The average composition of samples of odor isolates from individual preparations as well as from several different preparations of the same type—i.e., concurrent or noncurrent—was determined on the basis of all preparations which could be compared on a fair analytical basis (same gas chromatographic detector, column, and conditions). These results were then combined (traps plus distillate) to provide the rough estimations shown in Table IV. These data represent the best estimation of the composition of the total volatile odor concentrates from each processing method studied.

The nonirradiated isolates contained about equal amounts of 1-hexanal (22.86%) and a mixture of 1-octanal and acetoin (22.90%). Alcohols accounted for 32.25%. The irradiated isolates were similar, in that they contained about the same proportion of methional, but differed greatly in overall composition. The concurrently irradiated and distilled concentrates contained 55.24% aldehydes, 16.58% alcohols, and 3.67% hydrocarbons. The nonconcurrent isolates, on the other hand, contained 23.36% aldehydes, 18.30% alcohols, and 24.04% hydrocarbons.

Selection of those components of primary importance in producing irradiation odor was aided by the observation that when all fractions eluted after 1-heptanal (Table IV) were collected together in a single trap, the trap exhibited irradiation off-odor, particularly when samples of "distillate" (0°C.) isolate were separated, though weak off-odor was recognized when "traps" isolates (−78°C.) were examined. An arbitrary decision was thus made to consider as potentially indispensable components only those compounds present in irradiated "distillates" eluted after 1-heptanal. This automatically eliminated hydrocarbons from consideration, though they were recognized as products of the irradiation process.

Substances remaining for consideration are shown in Table V in percentages found in various beef isolates and in proportions calculated relative

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Table V. Relative Proportions Of Possible Indispensable Off-Odor Components in Nonirradiated and Irradiated Beef

	Nonirradiated		*Concurrent*		*Nonconcurrent*	
Compound	*% found*	*Relative amount*	*% found*	*Relative amount*	*% found*	*Relative amount*
1-Pentanol	5.60	14.0	1.80	4.9	1.08	0.88
Acetoin	22.90	57.2	9.93	26.8	17.11	14.0
1-Octanal						
1-Hexanol	1.98	4.9	~1.25	3.4	2.50	2.1
1-Nonanal	2.52	6.3	26.99	72.9	3.40	2.8
1-Heptanol	5.27	13.2	4.52	12.2	1.71	1.4
Methional	0.26	0.6	16.47	44.5	18.08	14.8
1-Decanal	0.34	0.8	~0.71	1.9	0.54	0.44
1-Octanol	0.99	2.5	~2.34	6.3	~2.82	2.3
1-Undecanal	1.05	2.6	0.60	1.6	0.48	0.39
Benzaldehyde	0.78	1.9	2.05	5.5	~2.82	2.3
Phenyl-acetaldehyde	0.40	1.0	0.37	1.0	1.22	1.0

to phenylacetaldehyde. Validity of the relative quantities was checked by rough determination of gas chromatographic detector response to the compounds shown in Table VI. No differences great enough to affect greatly the estimated composition of the odor concentrates, or of any synthetic mixtures which might be concocted, in any practical way, were noted.

Synthetic mixtures of irradiated beef components, based on the relative quantities given in Table V, were prepared by adding appropriate volumes of dilute aqueous ethanolic (5%) stock solutions of each compound to 100 grams of enzyme-inactivated ground beef slurried in 75 ml. of water. Stock solutions containing 238 p.p.m. were prepared by dissolving the gas chromatographically purified compound (13 mg.) in ethanol (3.7 ml.) and adding

Table VI. Rough Estimation of Detector Response to Certain Beef Components

		Response	
Compound	*Density, g./ml.*	*Per unit volume (found)*[a]	*Per unit weight (calc.)*[a]
1-Nonanal	0.827	1.00	1.21
1-Octanal	0.821	1.10	1.34
Phenylacetaldehyde	1.03	1.11	1.07
Benzaldehyde	1.05	1.11	1.06
1-Octanol	0.825	1.10	1.33
Methional	1.05	0.99	0.94
n-Dodecane	0.751	1.01	1.34
1-Dodecene	0.762	0.74	0.97
n-Undecane	0.741	0.67	0.91

[a] Relative response based on average peak area of three 1-μl. samples run on 20% Carbowax 20M column and detector used on beef odor concentrates.

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51.7 ml. of distilled water. They were kept in dropping bottles so that synthetic mixtures could easily be made based on the relative quantities shown in Table V.

The odor of the resulting "synthetic" irradiated beef slurries was compared with an equal quantity of freshly irradiated and slurried beef. Comparisons were made informally by persons experienced in sensory evaluation and familiar with irradiation off-odor. It was obvious almost immediately that some of the compounds contributed little or nothing to irradiation odor, while others affected it greatly. Trial and error mixing of compounds and concurrent odor evaluation led to the opinion that when added to a slurry of 100 grams of enzyme-inactivated beef in 75 ml. of distilled water, the following mixture caused very close approximation of irradiation off-odor.

		Relative Amount
Methional,	3.0 p p m.	20
1-Nonanal,	0.30 p.p.m.	2
Phenylacetaldehyde,	0.15 p.p.m.	1

Since the enzyme-inactivated beef used as substrate contributes volatile components of nonirradiated beef. the three substances added are not completely responsible for the resulting odor. They are, however, believed to be the most important contributors since even when added to water a good representation of irradiation odor is obtained. The quantities necessary in aqueous solution were

		Relative Amount
Methional,	5.0 p.p.m.	20
1-Nonanal,	0.5 p.p.m.	2
Phenylacetaldehyde,	0.25 p.p.m.	1

In both beef slurries and aqueous solutions the relative proportion of added components was methional (20): 1-nonanal (2): phenylacetaldehyde (1). Reference to the relative quantities shown in Table V indicates that the nonconcurrently processed isolate somewhat resembled the synthetic mixtures—i.e., methional (14.8): 1-nonanal (2.8): phenylacetaldehyde (1). The concurrent isolate was very different—methional (44.7): 1-nonanal (72.9): phenylacetaldehyde (1).

Statistical evaluation of the degree to which the "synthetic" sample (prepared by adding the above three compounds to nonirradiated enzyme-inactivated beef) differed from enzyme-inactivated irradiated beef, was determined. Using the score sheet given in Figure 4, panel members evaluated the odor quality of a standard sample (S) and two coded samples (one of which was identical with the standard) and indicated the degree of difference noted. Two sets of samples were tested in a single session. One had irradiated beef slurry as the standard sample (Set I, Table VII). The

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Compare the odor of the numbered samples with that of the reference sample "S." Indicate the degree of difference, if any, from the odor of "S" by checking the appropriate box opposite the term which best describes the degree of odor difference. Take as much time as you need. <u>Base your judgement on odor quality and not intensity</u>.

	Sample number		Score
	____	____	
None........................	☐	☐	1
Between None & Slight..........	☐	☐	2
Slight........................	☐	☐	3
Between Slight & Moderate......	☐	☐	4
Moderate......................	☐	☐	5
Between Moderate & Large.......	☐	☐	6
Large.........................	☐	☐	7
Between Large & Extreme........	☐	☐	8
Extreme.......................	☐	☐	9

Figure 4. Odor differences evaluation score sheet

other had a "synthetic" irradiated slurry as standard (Set II, Table VII). Panelists were allowed to take as long as they wished and to re-evaluate samples if they so desired. Samples (10 grams) were presented in glass-stoppered bottles (1 ounce) covered with aluminum foil, so that appearance did not affect the evaluation.

Results of evaluations of "synthetic" *vs.* irradiated samples are given in Table VII. Review of these data shows that even though sample S′ in each set was the same as the standard reference sample, panel members thought it differed to a slight degree in odor quality, since the means of all scores for S′ were 1.61 (Set I) and 1.74 (Set II). Observations of the two samples in each set were therefore paired, and the differences between means for each judge were determined. The hypothesis that no difference existed within each pair—i.e., that there was no difference between samples—was then evaluated using a t-test for correlated pairs. As shown in Table VII, this hypothesis had to be rejected for both sets. A significant difference did exist in both cases. However, the degree of these differences ($\bar{d}$ = 1.51 in Set I; $\bar{d}$ = 1.97 in Set II) was described by the panel to be less than slight. It was therefore concluded that major responsibility for irradiation odor in enzyme-inactivated beef slurries could be assigned to the presence of methional, 1-nonanal, and phenylacetaldehyde.

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Merritt, as a result of elegant analytical work on raw beef, has suggested (*8*, *10*, *12*) that the series of *n*-alkanes and 1-alkenes produced during irradiation are responsible for irradiation flavor. In our work no evidence has been found which supports this suggestion. The reason for this contradiction may be the different conditions used during irradiation. Merritt worked with raw beef which was irradiated in vacuum or an inert atmosphere. Our beef, on the other hand, had been partially cooked during enzyme-inactivation and then irradiated in the presence of air. It may also be that 1-nonanal, methional, and phenylacetaldehyde are not the only substances which when mixed in correct proportions give rise to typical irradiation odor.

Statistical evaluations of the threshold of significant effect of methional, 1-nonanal, and phenylacetaldehyde in enzyme-inactivated beef slurries were determined. A score sheet like the one shown in Figure 4 but providing for evaluation of three rather than two samples was used. Panel members evaluated the odor quality of a standard sample (S) and three coded samples, one of which was identical with the standard. The other two samples contained the test compound at increasing concentration levels.

Table VII. Degrees of Differences in Irradiation Odor

(85 evaluations, 17 judges)

Means of Differences from Standard S′		Differences	Means of Differences from Standard S′		Differences
Irradiated $\bar{X}_1$	*Synthetic* $\bar{X}_2$	*in Means* $(\bar{X}_2 - \bar{X}_1) = d_i$	*Irradiated* $\bar{X}_1$	*Synthetic* $\bar{X}_2$	*in Means* $(\bar{X}_2 - \bar{X}_1) = d_i$
SET I. STANDARD IRRADIATED SAMPLE			SET II. STANDARD "SYNTHETIC" SAMPLE		
1.00	2.20	+1.20	1.20	2.00	+0.80
2.50	3.25	0.75	1.22	4.00	2.78
1.66	3.00	1.34	1.33	1.33	0.00
1.00	2.00	1.00	1.00	3.00	2.00
2.00	2.57	0.57	2.00	3.29	1.29
1.83	3.66	1.83	3.83	3.66	−0.17
1.71	1.71	0.00	1.00	1.57	+0.57
1.33	4.00	2.67	1.50	4.17	2.67
2.66	1.66	−1.00	1.33	4.33	3.00
1.00	5.66	4.66	1.50	6.00	4.50
2.22	1.20	−1.02	1.60	2.40	0.80
2.00	2.14	0.14	1.43	2.86	1.43
1.00	3.50	2.50	1.50	4.33	2.83
1.00	5.50	4.50	1.00	5.83	4.83
1.00	5.50	4.50	1.00	5.50	4.50
2.00	1.77	−0.23	2.50	3.25	0.75
1.50	3.77	2.25	4.75	5.75	1.00
Mean 1.61	3.12	1.51[a] Difference	Mean 1.74	3.60	1.97[b] Difference

[a] For H_0, $\bar{d} = 0$; $t = 3.444$, $P \leq 0.01$.

[b] For H_0, $\bar{d} = 0$; $t = 5.13$, $P \leq 0.01$.

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A threshold of significant effect was considered to have been determined when a significant difference in odor quality from that of enzyme-inactivated nonirradiated beef slurry was noted. As before, a t-test for correlated pairs was used. The following quantities of methional, 1-nonanal, and phenylacetaldehyde were required.

Threshold of Significant Effect of Individual Compounds

Methional	6.0 p.p.m.
Phenylacetaldehyde	0.94 p.p.m.
1-Nonanal	>7.6 p.p.m.

Higher concentrations of 1-nonanal were not tried since 7.6 p.p.m. was already much greater than the amount needed in a mixture with methional and phenylacetaldehyde to cause irradiation off-odor to be recognized.

In view of the fact that irradiation odor was exhibited by mixtures which varied widely in the relative proportions of methional, 1-nonanal, and phenylacetaldehyde, it was somewhat puzzling to determine that irradiation odor production in enzyme-inactivated nonirradiated slurries depended on adding these compounds in 20:2:1 amounts, respectively. Although no attempt was made to determine whether these proportions could be varied within limits, the general impression was that variations probably would not result in irradiation odor. It is therefore suspected that factors such as masking effects by other components must be operative in the concurrently processed isolates to allow them to exhibit irradiation odor.

There is no doubt that widely differing sensory effects result from the presence, alone and in a mixture, of methional, 1-nonanal, and phenylacetaldehyde in meat slurries. This was illustrated by the observation that much greater amounts of these compounds were needed to cause a sensory difference when added individually to slurry than when present in a mixture.

	Individual Threshold, p.p.m.	*Quantity for Irradiation Odor, p.p.m.*
Methional	6.1	3.0
Phenylacetaldehyde	0.94	0.15
1-Nonanal	7.6	0.30

The significance of subthreshold concentrations as important contributors to aromas has been noted and discussed by others (*5, 6, 13*). It is clear that much more must be learned before the relationship among chemical structure, concentration, and the sensory effect of these compounds can be understood.

It is believed, however, that methional, 1-nonanal, and phenylacetaldehyde are the major contributors to the irradiation odor produced in enzyme-inactivated beef slurries.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch002

Acknowledgment

The authors thank Charles Merritt, Jr., U. S. Army Natick Laboratories, for use of the mass spectrometer and Phillip Issenberg, Department of Nutrition and Food Science, Massachusetts Institute of Technology, for obtaining the mass spectra.

The authors acknowledge with gratitude financial support from the U. S. Army Natick Laboratories which made the research possible. This paper reports research undertaken in cooperation with U. S. Army Natick Laboratories, Natick, Mass., under Contract No. DA 19–129–AMC–99 (N). The views or conclusions contained herein are those of the authors and do not necessarily reflect the views or endorsement of the Department of Defense.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch002

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RECEIVED November 9, 1965. Contribution 725, Department of Nutrition and Food Science, Massachusetts Institute of Technology.

3

Volatile Compounds Induced by Irradiation in Basic Food Substances

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch003

Irradiation flavor and odor in meat are believed to be caused by the volatile chemical compounds produced by radiation impact on the protein and lipid molecules. The volatiles from irradiated ground beef, pork, mutton, lamb, and veal as well as the volatile irradiation degradation products of several amino acids and proteins, animal fats, methyl esters of fatty acids, and triglycerides, have been analyzed by programmed cryogenic temperature gas chromatography to separate the complex mixtures obtained and rapid scanning mass spectrometry to identify the individually separated components. The results are compared with those obtained from irradiating meat itself and separate meat fractions, thus establishing the contribution of each fraction to the total. Mechanisms are postulated for the formation of the volatile components from each fraction.

The objective of these studies has been to discover the precursors of the volatile compounds produced in irradiated meat and the mechanisms by which they are formed. Three postulates based on sensory observations related to the production of irradiation odor have influenced the choice of irradiation conditions and technique, and all serve to delineate the problem more clearly. First, irradiation odor in raw meat is a characteristic property, is the same for beef, pork, lamb, and the other meats, and does not vary in type but only in intensity. Second, the odor is reproducible, and given radiation doses can produce about the same odor. Third, irradiation odor is the direct result of irradiation impact or damage and does not depend on the type of irradiation used or the presence of such variables as environmental water or oxygen.

The primary objective was to conduct a continuing investigation and to assemble facts relating to the analytical composition of volatile compounds produced in irradiated meat and of a number of model systems,

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

with the hope that this information would provide some understanding of the mechanism of formation of such compounds.

Experimental

The procedures for irradiation, collection, and analysis of the volatile compounds have all been described (*1, 2, 3, 5, 7, 9, 11*). A cryogenically programmed gas chromatograph coupled to a rapid scanning mass spectrometer provided for the analysis of the volatile components isolated from several irradiated meats and lipid substances (*6*). These studies have now been extended to include other component substances in order to acquire still further understanding of the source of the irradiation-induced volatile compounds from among the various meat constituents.

Table I. Substances Employed for Irradiation Studies

Whole Meats[a]	*Lipid*	*Protein*
Beef	Beef fat	Haddock (0.3% fat)
Veal	Butterfat[b]	Beef protein
Mutton	Triglycerides[a]	Oxytocin
Lamb	Fatty acid esters[a]	Amino acids
Pork		
	Cholesterol	
	Beef lipoprotein	

[a] (*6, 10*).
[b] (*4, 8*).

Table I summarizes the various meats, meat constituents, and other related substances which have been analyzed, including substances reported on previously (*6*) as well as those for which new data are given. The substances chosen are intended to provide a cross-section of the type of inherently related material from which volatile irradiation odor and flavor compounds might be expected to form. Thus, in addition to several whole meats, the volatile irradiation products from a number of protein and lipid substances have been analyzed. Among the lipid substances included are typical whole fats and separate moieties such as triglycerides, fatty acid esters, and cholesterol, as an example of a steroid. Among the proteinaceous substances included are a protein, a polypeptide, and some individual amino acids. Finally, beef itself has been separated into a protein, a lipid, and a lipoprotein fraction, and these have been separated, irradiated, and analyzed.

Results and Discussion

Although the results of the lipid and whole meat studies have been reported (*6, 10*), a brief review of the findings will help correlate the earlier work with the current studies of proteinaceous substances.

Analysis of the volatiles from irradiated ground beef, pork, mutton, lamb, and veal showed that the compounds formed are essentially the same in all the meats. All of the samples were irradiated at a dose of 6 megarads, and the volatiles produced show the presence of more than 80 compounds,

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch003

many in appreciable quantity. The analyses have been summarized (*1*). The controls, or unirradiated samples, showed the presence of only trace quantities of compounds except expected metabolites such as ethanol, acetaldehyde, or acetone.

The most abundant volatile components produced were the hydrocarbons. Figure 1 shows the distribution of *n*-alkanes in a sample of irradiated beef volatiles compared with a control. The small amounts of the hydrocarbons found in the unirradiated control are probably caused by oxidation of the meat fat during storage. The distribution of normal alk-1-enes corresponding to the normal alkanes, but in smaller quantity, was found to be uniform in all the irradiated meat samples. The olefin compounds together with the alkanes constitute approximately 90–95% of the total composition of the volatile constituents isolated. Alkanes constitute about 60% and alkenes about 40% of the total hydrocarbon content. Trace amounts of *n*-alkynes were also found among the compounds isolated from the various meats. Although hydrocarbons cannot be disregarded as potential sources of irradiation odor, at present it is more important to regard the formation of these compounds from the viewpoint of mechanisms than as odorants.

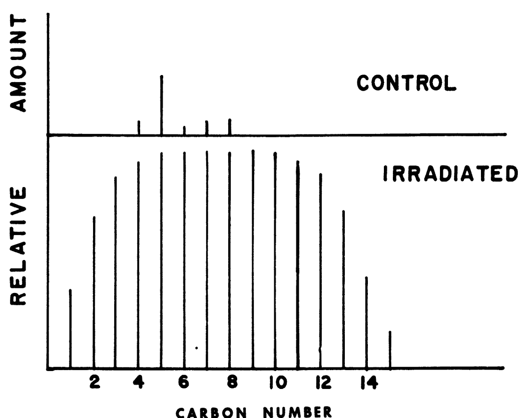


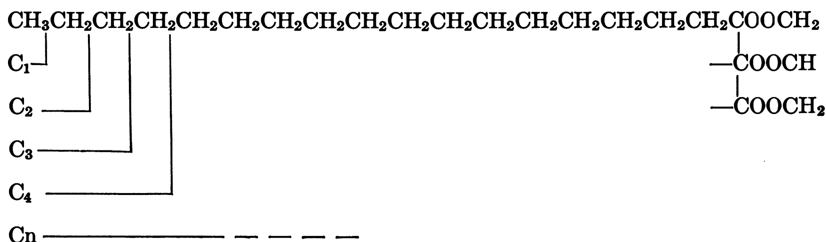
Figure 1. n-Alkanes identified in volatile compounds isolated from irradiated beef

It is now well established (*6, 10*) that the hydrocarbons, except possibly those having three or four carbon atoms, found in the irradiated meats can come only from the lipid. This hypothesis has been verified in earlier studies, when the volatiles from irradiated methyl oleate (*10*) were found to contain appreciable quantities of alkanes and alkenes, and now in more detail from studies of both triglycerides and fatty acid esters.

The mechanism of irradiation damage in lipids appears to be caused primarily by radiation-induced direct cleavage of carbon bonds to form

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch003

alkyl free radicals. Thus, scission of the bonds in the alkyl chains of glycerol tristearate, for example, with recombination or hydrogen termination

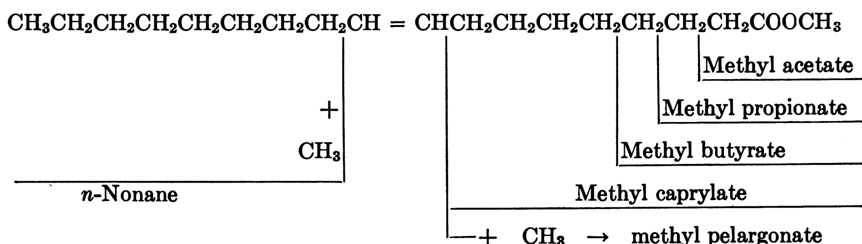


of the resulting alkyl free radicals can account for the *n*-alkanes from methane to heptadecane. All the alkanes to hexadecane have been found in good yield. If secondary collisions extracting a second electron occur, a similar homologous series of alkenes is predicted, and these also are detected in quantity. When the acids are unsaturated, such as oleic or linoleic, increased quantities of olefins are found.

Table II. Radiation Products Induced in Fatty Acid Esters and Triglycerides

	Methyl Stearate	*Methyl Oleate*	*Tristearin*	*Triolein*
n-Alkanes	$C_1 \rightarrow C_{16}$	$C_1 \rightarrow C_9$	$C_1 \rightarrow C_{13}$	$C_1 \rightarrow C_{10}$
n-Alkenes	$C_2 \rightarrow C_{10}$	$C_2 \rightarrow C_{10}$	$C_2 \rightarrow C_{10}$	$C_2 \rightarrow C_{10}$
Methyl esters	$C_2 \rightarrow C_{11}$	$C_2 \rightarrow C_9$		C_2 only
			Acetone	Acetone

The study of the radiation products induced in methyl oleate, methyl stearate, trioleate, and tristearin also supports the argument for direct bond cleavage. Data are given in Table II, and a representation of the process is indicated below.



Bond cleavage would be expected to lead to a homologous series of alkanes, alkenes, and esters to C_{10} if the fatty acid were oleate, but it would give higher homologs if the fatty acid were stearate. The principal products are, in fact, alkanes, alkenes, and a homologous series of methyl esters. The highest member of the alkane series found in irradiated methyl oleate

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch003

is *n*-nonane, and the highest methyl ester is methyl pelargonate—i. e., the C_9 acid—whereas hexadecane is found in methyl stearate and tridecane in tristearin. No methyl esters are found, however, among the radiation products in tristearin or triolein.

Most of the other products found in irradiated meat volatiles except those containing sulfur or aromatic rings may also be accounted for by mechanisms associated with alkyl free radical formation in the fat. Oxygenated compounds are far less abundant than hydrocarbons, but appreciable amounts of a homologous series of *n*-aliphatic alcohols up to hexanol are found. Of these, only ethanol is detected in the unirradiated controls. Since the water content of meat averages nearly 60%, the formation of alcohols may be thought to occur by reaction of the alkyl free radical with water. Such a mechanism is supported by the fact that only traces of alcohols are found in irradiated dry butterfat and were undetected in irradiated triglycerides or methyl esters of fatty acids.

The aldehydes and ketones are least abundant of all the compounds found which may be considered as derived from the fat. The carbonyl compounds are probably produced by an indirect route, which is most likely similar to that involved in autoxidation of a fat. The alkyl free radical can absorb oxygen, form a hydroperoxide, and then follow the many decomposition paths which are familiar in the oxidation chemistry of fats. The more abundant aldehydes found are unsaturated, which further agrees with the hypothesis that they are derived from the decomposition of hydro-

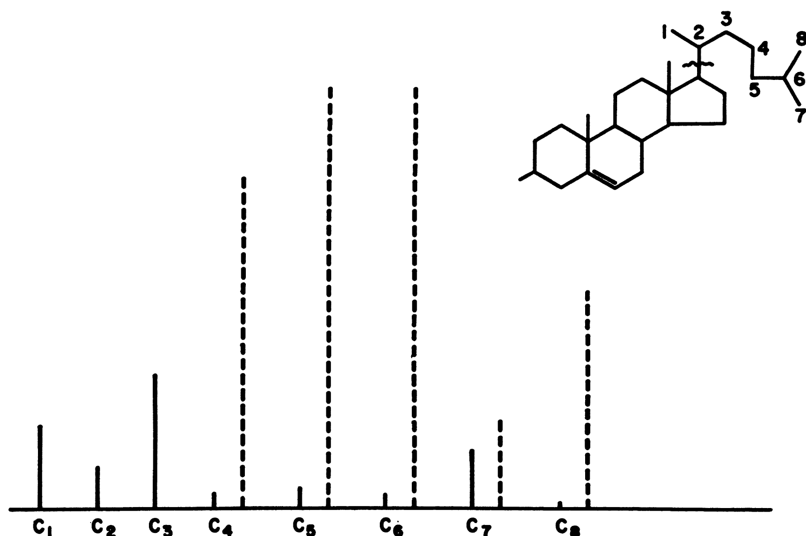


Figure 2. Volatile irradiation products of cholesterol ——— *Normal alkanes* – – – *Isoalkanes. Hypothetical cleavage of alkyl side chain indicated on structural diagram*

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch003

peroxides. This mechanism is also supported by studies of dry butterfat irradiated in the absence of air (*8*). Carbonyl compounds were found only in trace amounts.

Although minor, the contribution from steroid substances has been generally neglected. Figure 2 shows the compounds isolated from a sample of cholesterol irradiated at 6 megarads. The principal products were a series of normal alkanes from C_1 to C_7 and a series of isoalkanes from C_4 to C_8. The relative abundance of the iso compounds compared with the normal compounds is shown by dashed lines. The origin of these series of compounds is readily deduced to be the result of cleavage of the alkyl side chain of the cholesterol molecule.

Table III. Miscellaneous Compounds in Irradiated Meat Volatiles

Sulfur dioxide	Dimethyl sulfide	Benzene
Carbonyl sulfide	Methyl ethyl sulfide	Toluene
Hydrogen sulfide	Dimethyl disulfide	Phenol
C_1–C_6 thiols	Diethyl disulfide	*p*-Cresol

In meats, of course, there are components which arise from the protein which cannot be present in the products from pure fat. Table III shows some of the sulfur compounds and aromatic compounds which are also found in irradiated meats. Many of these can be postulated as arising from direct bond cleavage of amino acid moieties. Benzene and toluene may come from phenylalanine and phenol and *p*-cresol from tyrosine. Recent studies have been directed to considering the origin of some of the compounds from proteinaceous substances. Some of the sulfides, disulfides, and mercaptans can derive directly from cysteine or methionine, but those containing more than two carbon atoms in a chain require more than a superficial explanation. In order to evaluate the contribution of the volatiles from the protein as well as the lipid constituents of meat, volatile components produced in various protein substances have also been analyzed.

The principal components found in haddock flesh irradiated at 6 megarads are summarized in Table IV. Haddock was chosen because it was a

Table IV. Volatile Compounds Isolated from Irradiated Haddock Flesh

Sample size 500 grams. Fat content 0.3%

Methane (t)[a]	Methyl mercaptan (l)
Propane (s)	Dimethyl sulfide (l)
Butane (s)	Dimethyl disulfide (l)
Butene (t)	Ethanol (s)
Benzene (l)	Acetone (m)
Toluene (l)	Methyl ethyl ketone (m)

[a] (l) large, (m) moderate, (s) small, (t) trace.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch003

convenient source of animal protein which is relatively fat-free. The odor of the irradiated haddock flesh and of the corresponding total condensate were typically meaty in character, not fishy, as was the odor of the unirradiated haddock. The meaty odor in the irradiated haddock seemed to resemble that of fresh meat and did not have any of the typical attributes of irradiation odor.

The major components identified among the volatiles produced in haddock upon irradiation are benzene and toluene and the sulfur compounds. These compounds may be expected from the radiation-induced degradation of protein. The only carbonyl compounds found are acetone and methyl ethyl ketone, and these are present in only moderate amount. Trace quantities of low molecular weight hydrocarbons were also found. The detection of hydrocarbons, even in trace amounts, led us to question whether their origin was in the protein or in the small amount of fat present in the haddock.

It thus seemed that the origin of the various components in meat volatiles could best be established by analyzing irradiation-induced compounds in meat protein and meat fat separately. Accordingly, a 500-gram sample of meat, the same size of sample normally used in irradiation studies of whole meat, was separated into a protein, a lipid, and a lipoprotein fraction by means of a methanol-chloroform extraction of the fat. The dry, air-free, fractions were then irradiated separately with 6 megarads of gamma radiation in the manner used for whole meat. The analytical results (Table V) show clearly that mainly sulfur compounds and aromatic hydrocarbons are formed in the protein fraction, whereas mainly aliphatic hydrocarbons are formed from the lipid. The lipoprotein fraction produced, as expected, both aliphatic hydrocarbons and sulfur compounds. Only the lipoprotein fraction had a characteristic irradiation odor.

Table V. Volatile Compounds Isolated from Meat Substances

Protein	*Lipid*	*Lipoprotein*
Methyl mercaptan (l)	C_1–C_{12} *n*-alkanes (l)	C_1–C_{14} *n*-alkanes (l)
Ethyl mercaptan (s)	C_2–C_{15} *n*-alkenes (l)	C_2–C_{14} *n*-alkenes (l)
Dimethyl disulfide (m)	C_4–C_8 iso-alkanes (s)	Dimethyl sulfide (s)
Benzene (m)	Acetone (m)	Acetone (m)
Toluene (m)	Methyl acetate (t)	
Ethylbenzene (2)		
Methane (s)		
Carbonyl sulfide (s)		
Hydrogen sulfide (s)		

To acquire further insight into the mechanism of the formation of these compounds, particularly of the protein-derived components, the radiolysis of several amino acids was studied. In all the amino acid studies, solutions or slurries of the acid were used in amounts corresponding to the

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch003

average amount present in 500 grams of meat, the usual size of a meat sample. The results are in good agreement with the actual observations for meat protein—i. e., sulfur and aromatic moieties seem to be most subject to radiation cleavage. The results of the analyses are shown in Table VI. (Tyrosine, leucine, and glutamic acid were also studied, but no volatile compounds other than carbon dioxide were found.) The major and universal product of amino acid irradiation is carbon dioxide, which is produced in large quantities. Decarboxylation is apparently the major effect induced by the radiation. The other volatile products, if any, are the expected decomposition products which may arise from cleavage of the side chain moieties.

Table VI. Volatile Compounds Produced in Some Irradiated Amino Acids

Amino Acid	*Major Components*[a]
Cysteine	Sulfur dioxide (l)
	Hydrogen sulfide (m)
Cystine	Sulfur dioxide (s)
	Carbonyl sulfide (m)
	Carbon disulfide (m)
	Dimethyl disulfide (t)
Methionine	Methyl mercaptan (s)
	Dimethyl sulfide (m)
	Dimethyl disulfide (l)
Phenylalanine	Toluene (1)
Arginine	Unidentified nitrogen compound (m)

[a] Carbon dioxide present in large amount in all samples.

Thus, among the sulfur amino acids, hydrogen sulfide is a likely derivative of cysteine, and dimethyl disulfide is a likely derivative of cystine. The formation of dimethyl sulfide and dimethyl disulfide from methionine is readily deduced from expected recombinations of thiomethyl and methyl free radicals. Dimethyl disulfide is the major product.

Other amino acids were chosen to typify an aromatic, a basic, a neutral, and an acidic amino acid. Of these, only phenylalanine proved to be radiosensitive at the doses and concentration used here.

The volatile products from irradiation of oxytocin were examined to see if any indication of destruction of peptide bonds could be observed. The products found were mainly short-chain hydrocarbons and were readily explained by cleavage of the side chains from the leucyl and isoleucyl constituents. No evidence of rupture of peptide bonds is seen.

In all amino acid and peptide radiolysis, sulfur dioxide and carbonyl sulfide were found in varying amounts, but at present no explanation for the formation of these compounds can be offered.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch003

Conclusions

The data thus far obtained support the simple hypothesis that radiation products are primarily the result of direct bond cleavage. The main products of irradiation of dry oxygen-free lipid substances are the homologous series of *n*-alkanes, *n*-alkenes, and traces of *n*-alkynes. Sterols give mainly normal and isoalkanes from cleavage of the alkyl side chain. Proteins and peptides show little evidence of rupture of the peptide bond, and the main products are from cleavage of the side chains on end groups. The amino acids with aromatic rings or with sulfur groups tend to be most radio-sensitive.

There is evidence that some compounds found in meat are produced by an interaction between phases. For example, hexyl mercaptan or ethyl butyl disulfide would seem to come from free radicals originating in part in the protein and in part in the lipid portions of the meat. Although the current work has seemingly provided greater insight into the mechanisms of irradiation damage in meat, it has also raised more questions. Further studies will be directed toward relating the effects of irradiation on model systems of fat and protein substances in mixtures to try to clear up some of the questions of phase interactions.

Acknowledgment

The authors are indebted to M. L. Bazinet for his contributions to the mass spectral analyses.

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RECEIVED November 9, 1965.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch003

4

Effect of Irradiation Temperature and Processing Conditions on Organoleptic Properties of Beef and Chemical Yields in Model Systems

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch004

The irradiation flavor intensity of enzyme-inactivated beefsteaks decreases linearly with irradiation temperature in the range +20° to −196°C. The rate of decrease varies with the irradiation dose. A large temperature effect on the yields of primary radiochemical reactions of amino acids and peptides in aqueous solutions was found in chemical studies conducted in our laboratory. A process variable study on enzyme-inactivated steaks showed that an organoleptically superior radiation-sterilized steak can be produced by irradiating at liquid nitrogen temperatures in vacuum and slowly warming to room temperature for storage.

Radiations with energies above 10 m.e.v. induce detectable levels of radioactivity in food products (*33*). However, radiations in the energy range proposed for food irradiation, 0.1–10 m.e.v., lose their energy almost exclusively by transferring it to the electrons of the irradiated material, producing electronically excited or ionized molecules.

Chemical effects follow as a result of the transfer of some of the energy of electronic excitation or ion neutralization into vibrational energy sufficient to break bonds. Most of these chemical events result in the formation of free radicals, which may combine with each other or react with other molecules in their vicinity, the ultimate chemical change produced in the system being determined by the proximity of the activated molecules or radicals to each other and the activation energies of possible competing reactions.

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

Ionizing radiations may affect molecules or microorganisms either directly by a "hit" on the target area or indirectly via reactions of these molecules with the free radicals formed when the radiation is absorbed by a solvent. To a great extent, the microbes in food which cause spoilage are destroyed through direct action or hit of the particles of energy at or near sensitive areas in the cell (*18*). As a further generality, the chemical changes which occur when radiation reacts with food products are produced by the indirect action of the radiation's reacting with water and causing the formation of free radicals and activated molecules (*19*). These reactive entities then produce the chemical changes observed—formation of foreign flavor compounds, changes in texture, odor, color, and so forth—all of which are generally called side reactions.

The effect of irradiation temperature on these indirect or side reactions can be large while the effect of temperature on direct-hit processes such as the destruction of *C. botulinum* spores is generally small (*17, 19*). For example, the rates of carbonyl and mercaptan production in gamma-irradiated methionine and glycylmethionine solutions at liquid nitrogen temperatures are less than 10% of those at room temperature while the effectiveness of gamma radiation in the destruction of *C. botulinum* spores is at least 75% as great at liquid nitrogen temperatures as at room temperature. The most serious consequence of the side reactions which occur in the radiation sterilization of meats is the development of a characteristic irradiation flavor and odor.

Several laboratories (*7, 9, 10, 20, 28*) have reported that the intensity of the irradiation flavor produced in meats receiving an equal dosage of irradiation is decreased when the irradiation is carried out at low temperatures (down to −196°C.). The limited data from early experiments in low temperature irradiation (*7, 9, 20*) working at temperatures down to −80°C. suggest that this effect is caused primarily by irradiation in the solid state. These data suggest a sudden decrease in irradiation flavor in passing through the freezing zone, with only slight improvement at lower temperatures. However, in our recent studies of the effect of irradiation temperature on irradiation flavor intensity (*30, 31*), the irradiation flavor intensity (as measured by a trained panel) was found to decrease linearly with temperature from +20° to −196°C., both at low (3 megarads) and high (6 megarads) irradiation doses, the effect being larger at the higher dose. These results suggest the operation of a temperature effect more complex than simple entrapment of primary free radicals in a solid matrix. Taken together with observations that free radicals observed in proteins irradiated at low temperatures (−196°C.) differ from those observed at room temperature, they suggest an interesting and important area of irradiation chemistry which has received little attention from food chemists.

The only reported attempt to determine the effects of irradiation temperature on radiation chemical yields from meats is the work of Merritt

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch004

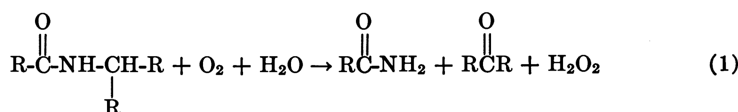
(*41*), who found that certain gas-liquid chromatographic peaks from irradiated beef volatiles were sharply diminished when irradiation was done at low temperatures.

A number of investigators (*2, 5, 15, 21, 34, 41, 42, 48, 52, 53*) have tried to isolate and to characterize the chemical compound or compounds which give rise to irradiation flavor in meat or to correlate irradiation flavor scores with the production of specific compounds or types of compounds during the irradiation of meat or meat fractions (*3, 4, 32, 44, 49, 50*). These investigations have indicated some probable and some improbable sources of irradiation flavor and the order of magnitude of the concentration of the compounds responsible for irradiation flavor. Wick *et al.* (*53*) have offered impressive chemical and organoleptic data connecting the 20:2:1 ratio of methional, 1-nonanal, and phenylacetaldehyde found in irradiated beef at the parts per million level with typical irradiation odor.

Merritt (*42*) has carefully studied the yields of hydrocarbons on irradiation of meat and meat components and proposed mechanisms for their formation during irradiation. Despite this recent progress, the chemical characterization of irradiation flavor in meats is far from complete. Little is known about the radiation-induced chemical processes giving rise to the compounds proposed as important to irradiation flavor or the identity of the chemical precursors of these compounds. However, irradiation flavor in beef appears to be associated largely with the protein constituents in meat (*21*).

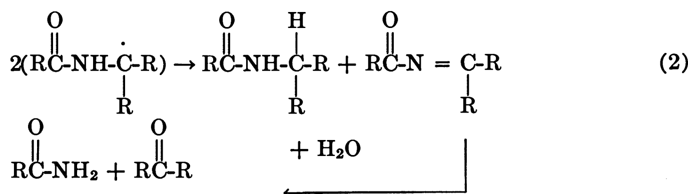
The major radiochemical reactions in aqueous protein systems are the result of free radical attack at the peptide bond, resulting in the formation of a carbonyl and amide or ammonia (*14*).

Garrison, Bennett, and Cole (*14*) propose the over-all primary reaction:



$$R\text{-}\overset{O}{\overset{\|}{C}}\text{-}NH\text{-}\underset{R}{\underset{|}{C}}H\text{-}R + O_2 + H_2O \rightarrow R\overset{O}{\overset{\|}{C}}\text{-}NH_2 + R\overset{O}{\overset{\|}{C}}R + H_2O_2 \quad (1)$$

for oxygenated solutions and a free radical disproportionation reaction followed by hydration for oxygen-free solutions.



$$2(R\overset{O}{\overset{\|}{C}}\text{-}NH\text{-}\underset{R}{\underset{|}{\dot{C}}}\text{-}R) \rightarrow R\overset{O}{\overset{\|}{C}}\text{-}NH\text{-}\underset{R}{\underset{|}{\overset{H}{\overset{|}{C}}}}\text{-}R + R\overset{O}{\overset{\|}{C}}\text{-}N = \underset{R}{\underset{|}{C}}\text{-}R \quad (2)$$

$$\xrightarrow{+H_2O} R\overset{O}{\overset{\|}{C}}\text{-}NH_2 + R\overset{O}{\overset{\|}{C}}\text{-}R$$

For proteins and peptides having sulfydryl or mercaptal side chains, free radical disproportionation reactions such as the ones reported by

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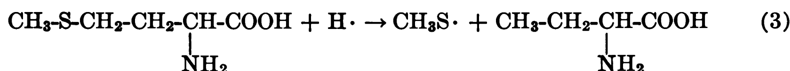
Tappel *et al.* for methionine (*49*) may also be important, producing hydrogen sulfide, mercaptans, and disulfides.



$$CH_3\text{-}S\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH + H\cdot \rightarrow CH_3S\cdot + CH_3\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH \quad (3)$$

Actually the irradiation chemistry of even simple amino acids and peptides is much more complex than these equations imply, and many other radiochemical products have been reported (*13, 14, 49*). These include glyoxylic acid, formaldehyde, hydrogen peroxide, carbon dioxide, succinic acid, aspartic acid, diaminosuccinic acid, methylamine, methane, dimethyl mercaptan, α-aminobutyric acid, methionine sulfoxide, methinonine sulfone, methionine sulfoxime, homocysteic acid, and several unidentified nitrogen compounds (*46, 51*).

The study of radiochemical yields in such analytically difficult and variable systems as meat proteins is a complex problem. Fortunately, however, both chemical (*4, 12, 23, 46, 49*) and electron spin reasonance studies (*6, 16, 22–25, 43*) by several workers have shown that the major radiochemical reactions in proteins closely parallel those of simple peptides and amino acids, justifying the use of these simpler systems for preliminary radiochemical investigations.

EFFECT OF TEMPERATURE ON CHEMICAL YIELDS IN AMINO ACIDS AND PEPTIDES

To gain some insight into the chemical effects of irradiation temperature on proteins, 2% solutions of glycine, glycylglycine, methionine, and glycylmethionine, both oxygen-free and saturated with oxygen, were irradiated at temperatures from +20° to −196°C. with doses up to 10 megarads and the ammonia, carbonyls, and mercaptans were measured.

Glycine, methionine, glycylglycine, and glycylmethionine solutions were selected for study primarily on the basis of electron spin resonance studies of irradiated proteins (*16, 22, 23, 43*), peptides (*6*), and amino acids (*25*) which show:

Proteins irradiated at room temperature exhibit two types of resonance structure (*16*), one similar to that of the peptide glycylglycine and the other a sulfur resonance similar to that found on irradiating cysteine.

Irradiation of proteins, peptides, and amino acids at −196°C. produces radicals different from those at room temperature. No sulfur radicals are observed at this temperature.

On heating proteins irradiated at −196°C. stepwise to room temperature, sulfur radicals appear (at temperatures above −120°C. using a 4-minute heat treatment), and nonsulfur radicals thermalize to room temperature–stable forms.

Both intra- and intermolecular radical transfer processes occur (*22, 23*) with transfer of the unpaired electron to a "free" sulfhydryl or disulfide group—an important process, particularly at higher temperatures.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch004

Radical yields of the same order of magnitude are observed for a number of amino acids and proteins, and heat denaturation of proteins in solution does not significantly alter these yields.

These four compounds were also selected because they were commercially available in pure form and reasonably soluble in unbuffered solutions at room temperature. In addition, the radiation chemistry of glycine, glycylglycine, and methionine has been studied at room temperature (*4*, *5*, *6*, *12*, *36*, *49*, *50*, *51*). Also, sulfur-containing amino acids have been suggested by chemical-irradiation flavor correlation studies (*4*, *21*, *38*, *44*, *49*, *50*, *53*) as being related to irradiation flavor.

The decision to study each of these compounds in both oxygenated and oxygen-free solutions was based on Hannan's observation (*20*) that irradiation flavor production at both 20° and −80°C. was affected by oxygen level and Henriksen's observation (*24*) that the presence of oxygen hindered the formation of sulfur-free radicals. Helium was chosen as the inert gas to maintain the oxygen-free solutions rather than nitrogen to avoid any possibility of producing ammonia or nitrogen acids from this source during the irradiation.

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Experimental Procedures

Preparation and Irradiation of Solutions. Glycine, methionine, glycylglycine, and glycylmethionine were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, and used without further purification. All samples dissolved completely in deionized distilled water to give colorless solutions, except glycylmethionine which had a pale yellow color. Each solution was within ±0.2 pH unit of its isoelectric pH (5.5 to 6.0).

Solutions (2%) of each compound in deionized distilled water were conditioned by bubbling either water-saturated helium or oxygen through the stirred solution for 2 hours and then dispensed under the appropriate protective atmosphere into 2-ounce polyethylene bottles (with tight-sealing screw-cap tops). The solutions were then rapidly frozen and sealed upright in purged metal cans for irradiation. Each can contained one bottle of each of the four solutions. The canned samples were held in dry ice (−80°C.) storage until irradiation time.

The canned, frozen samples were shipped to and returned from the cobalt-60 irradiation facility at the U.S. Army Natick Laboratories, Natick, Mass., packed in dry ice, and allowed to remain at −80°C. for 2 weeks, then at −20°C. for 2 weeks before thawing for analyses. This was done to permit reaction and slow thermalization of long half-life radicals at low temperatures, as well as to prevent spillage and thermal deterioration.

Analytical Procedures. Carbonyls were determined using a slight modification of the method of Lappin and Clark (*35*). A developed sample from each irradiated solution was scanned on a Beckman DK-2 ultraviolet spectrometer. Maximum absorptions occurred between 440 and 448 mμ. The absorption spectra were similar to that of pyruvic acid. Readings were made on a Beckman DU ultraviolet spectrometer at 446 mμ, using pyruvic acid as a standard.

Ammonia was determined by nesslerization, using a procedure taken from Higuchi (*26*) in which 2-ml. aliquots were mixed directly with reagents for color development and read at 426 mμ.

Mercaptans and hydrogen sulfide were determined using Tappel's (*50*) modifications of the methods of Sliwinski and Doty (*47*) and Marback and Doty (*38*). A 0.1M amine solution was used for the mercaptan analysis since it was found to give as much color development in the concentration range used as the specified 0.5M amine reagent and a much lower reagent blank reading.

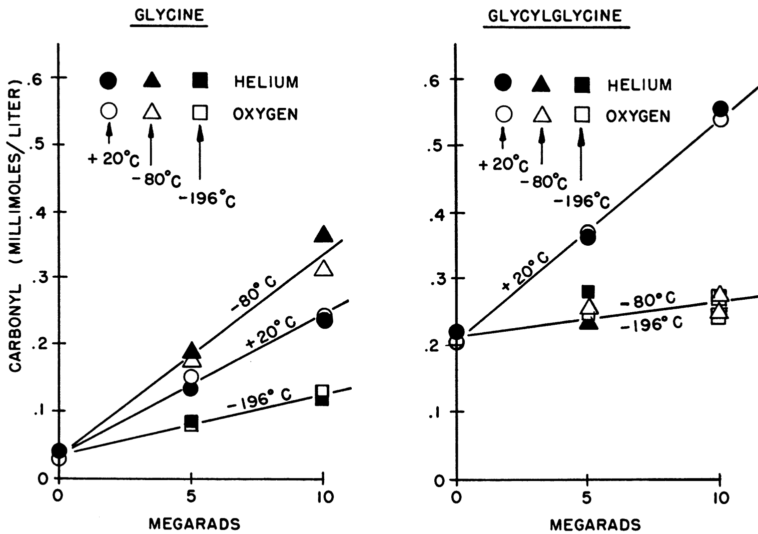

Figure 1. Yield of carbonyls from 2% glycine and glycylglycine solutions as a function of temperature and dose

Experimental Results

The experimental data are presented in Figures 1, 2, 3, 4, and 5 as isothermal plots of yield *vs.* dose, from which the radiation yield G values (molecules produced per 100 electron volts of absorbed energy) in Table I were calculated. In establishing these curves of isothermal yield *vs.* dose, two rules were used: straight-line fits were used unless major curvature was evident, and where no effect of oxygen on yields was evident, a single line was fitted through both sets of points. In general, the points fell on the straight lines within the precision of measurement.

Methionine and glycylmethionine interfered in the ammonia determination, and although color developed, no ammonia yields are reported for these compounds. A fine precipitate also appeared on nesslerizing some of the irradiated glycylglycine solutions, which prevented their analysis. The ammonia yields for glycine solutions were so low they were at the

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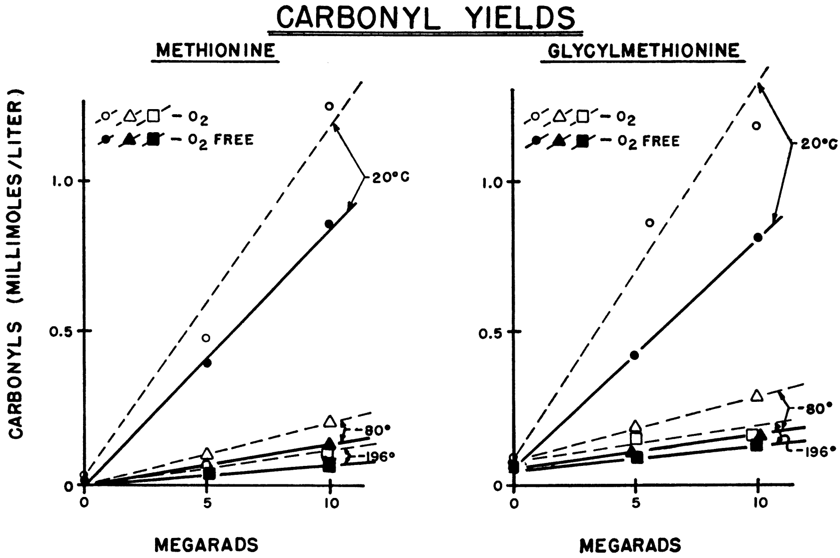

Figure 2. Yield of carbonyls from 2% methionine and glyclymethionine solutions as a function of temperature and dose

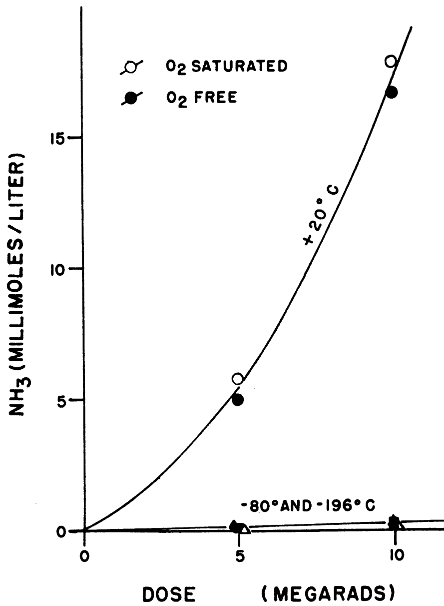

Figure 3. Yield of ammonia from 2% glycylglycine solution as a function of temperature and dose

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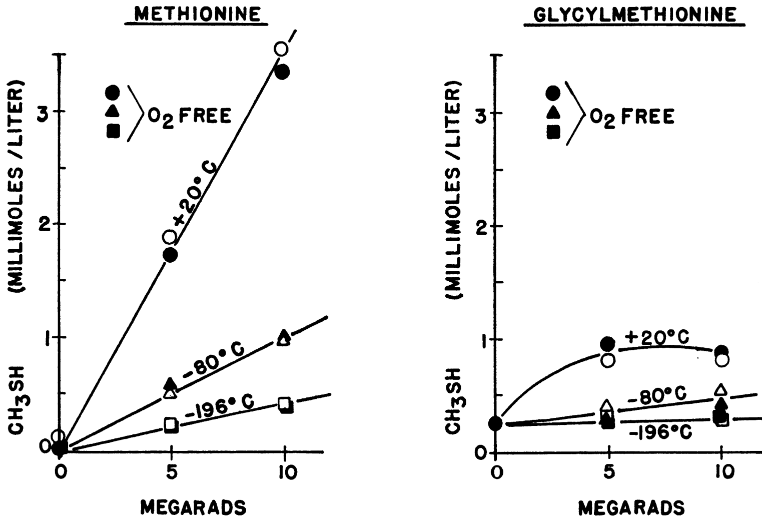

Figure 4. Yield of mercaptans from 2% methionine and glycylmethionine solutions as a function of temperature and dose

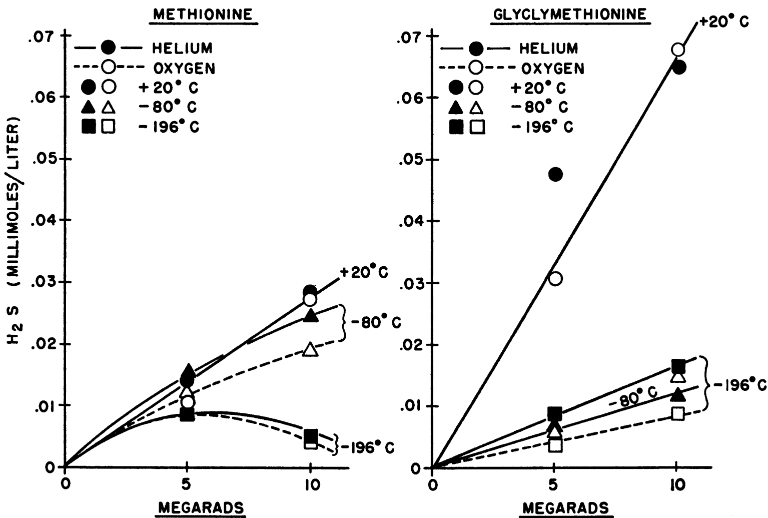

Figure 5. Yield of hydrogen sulfide from 2% methionine and glycylmethionine solutions as a function of temperature and dose

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Table I. G Values[a] × 100 Observed at Irradiation Doses up to 5.0 Megarads in 2% Amino Acid or Peptide Solutions Saturated with Helium (O_2 free) or Oxygen at Three Temperatures

	Temperature, °C.					
	Oxygen Free			*Oxygen Saturated*		
Solution	*+20*	*−80*	*−196*	*+20*	*−80*	*−196*
			G (Carbonyls) × 100			
Glycine	2.5	3.5	1.1	2.5	3.5	1.1
Methionine	8.9	1.5	0.9	12.4	2.2	1.1
Glycylglycine	3.8	0.7	0.6	3.8	0.6	0.6
Glycylmethionine	8.4	1.0	0.8	13.7	2.4	1.4
			$G (NH_3)$ × 100			
Glycine	0.3	0.6	0.01–0.03	0.2	0.9	0.02–0.07
Glycylglycine	126–186[b]	3.8	1.8	126–186[b]	1.7	—
			G (Mercaptans) × 100			
Methionine	38	11.	3.8	39.	11.	3.8
Glycylmethionine	23[c]	2.5	0.8	23.[c]	2.5	0.8
			$G (H_2S)$ × 100			
Methionine	0.32	0.32	0.23[c]	0.32	0.29	0.23[c]
Glycylmethionine	0.74	0.14	0.18	0.74	0.14	0.10

[a] Molecules produced per 100 e.v. of absorbed energy.
[b] Varies with irradiation level, G increases with dose.
[c] Initial slope, G decreases with dose.

limits of detection for the method (10^{-6} mole per liter) and are only estimates. Hence, they were not plotted. However, approximate G values are reported in Table I.

Discussion

Comparison with Literature Values at +25°C. The carbonyl G values at room temperature (+25°C.) generally agree with those reported in the literature for similar conditions (Table IV).

Garrison (*12*, *14*, *51*) and Maxwell (*39*, *40*) consistently report much higher G values for carbonyl and ammonia production (G = 3 to 5) than those in Table II. Their values are based on extrapolations back to zero dose (*39*) or referred to the ammonia plus amine G value at zero dose obtained by Maxwell, Peterson, and Sharpless (*39*) and are intended to reflect initial radical yield values important in theoretical treatments (*12*, *51*). Examining the data in these references shows that G values at higher doses approach those reported in Table II. For example, Maxwell (*40*) shows an observed G value at 1 megarad of about 0.4 for carbonyl production compared with the zero-dose extrapolation value of about 4.5.

The ammonia production in glycine solutions (G = 0.003) at room temperature was much lower than expected for this concentrated a solution (0.27M). G values of 0.1 to 3.0 were expected, based on the literature. However, Barron (*1*) has reported no detectable ammonia production in

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Table II. Carbonyl *G* Values Reported by Various Investigators for 5- to 10-Megarad Doses in 0.01 to 0.27*M* Neutral Aqueous Solutions at 20° ± 5°C.

Investigator	*Dose, Megarads*	*Compounds* Glycine	Glycylglycine	Methionine
This report				
O_2 free	10	0.025(0.27*M*)	0.038(0.15*M*)	0.089(0.134*M*)
O_2	10	0.025(0.27*M*)	0.038(0.15*M*)	0.124
Bellamy				
Air (*5*)	5	0.03 (0.01*M*)	0.035(0.01*M*)	
Air (*4*)	10			0.46 (0.1*M*)
Tappel (*50*)				
N_2	5	0.21 (0.1*M*)	0.13 (0.2*M*)	0.15 (0.1*M*)
		0.014(0.1*M*)		
Duran (*11*)				
N_2	10	0.13 (0.1*M*)		0.14 (0.1*M*)
	2	0.03 (0.1*M*)		0.22 (0.1*M*)
Liebster (*36*)				
O_2	10		0.02 (0.05*M*)	
N_2	10		0.01 (0.05*M*)	

$10^{-4}M$ glycine solutions and a *G* value of only 0.13 in a 0.1*M* oxygen-saturated solution using a dose of 0.05 megarad.

Effect of Oxygen. The presence of oxygen had no significant effect on carbonyl or ammonia yields in either glycine or glycylglycine solutions or on the mercaptan yield in methionine or glycylmethionine solutions. It increased the carbonyl yield by 70% in glycylmethionine and by 40% in methionine at each of the three temperatures. Since no corresponding decreases are seen in mercaptan or hydrogen sulfide yields, these do not appear to be competing processes.

Effect of Temperature. The effects of temperature on chemical yields are summarized in Table III in terms of percentage of +20°C. yields observed at −80° and −196°C.

Carbonyl yields for glycylglycine, methionine, and glycylmethionine are sharply reduced at −80°C., and a slight additional reduction occurs at −196°C. For glycine a significant increase in carbonyl yield occurs at −80°C., followed by a large decrease on reducing the temperature to −196°C. The same pattern holds for the data on ammonia yields, as one would expect from Equations 1 and 2. The increase at −80°C. suggests an effect of phase change while the opposed temperature effect at −196°C. suggests that a different reaction mechanism is controlling at −196°C. consistent with observations from electron spin resonance studies that different stable-free radicals are observed below −150°C. for glycine. The low carbonyl yields found for methionine and the peptides at −80°C. indicate that the "low temperature" radicals may still be the stable forms at

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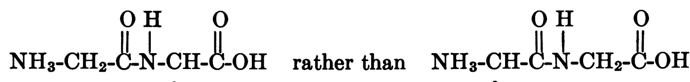
−80°C. Data at temperatures in the range 0° to −80°C. are needed to determine the temperature at which the transition from low temperature to room temperature radicals occurs.

Mercaptan yields from methionine and glycylmethionine show a temperature effect similar to that on carbonyl yields. Since electron spin resonance studies show that sulfur radicals are not observed at −196°C. in amino acids or proteins, it is likely that the small yields observed, presumably by way of Equation 3, are produced during the warming up period as stable "low temperature" radicals are converting to room temperature–stable forms. The higher mercaptan yield from methionine than from glycylmethionine at −80°C. may reflect greater thermal stability for the "low temperature" radical forms in the peptides than in the amino acids—an effect also suggested by the relative carbonyl and ammonia yields of glycine and glycylglycine discussed previously.

Table III. Effect of Irradiation Temperature on Yields of Irradiation Products in 2% Amino Acid and Peptide Solutions

	Percentages of Yields (G Values) at 20°C. Observed at Indicated Solution Temperatures							
	Glycine		*Glycylglycine*		*Methionine*		*Glycylmethionine*	
	Temperature °C.							
Products	*−80*	*−196*	*−80*	*−196*	*−80*	*−196*	*−80*	*−196*
	Oxygen Free							
Carbonyls	141	44	17	17	17	10	12	9
NH_3	210	4–11	2–3	1–1.5				
Mercaptans					30	10	11	3
H_2S					102	73	18	24
	Oxygen Saturated							
Carbonyls	141	44	17	17	18	9	17	10
NH_3	400	9–30	1–1.4	—				
Mercaptans					30	10	11	3
H_2S					91	73	18	13

Amino Acid *vs.* Peptide Yields. The peptides show greater yield reductions at low temperatures than do the corresponding amino acids, suggesting a stabilizing effect on the radicals produced at low temperature. Yields from glycylmethionine more closely resemble the yields from methionine than from glycine. This is consistent with the results of an electron spin resonance study of crystalline glycylglycine (*6*), which showed the structure of the radical produced at 20°C. to be:



EFFECT OF PROCESSING VARIABLES ON ORGANOLEPTIC ACCEPTABILITY OF BEEFSTEAKS

The effects of seven processing variables which, on the basis of previous literature reports (*7*, *27*) and our chemical studies on model systems, might affect the irradiation flavor intensity or consumer acceptance of irradiated steaks were evaluated in statistically designed and analyzed experiments. Steaks were then prepared using the processing conditions shown to be optimum by these experiments and evaluated for consumer acceptance and storage stability.

Experimental Procedures

Sample Preparation. Broiled beefsteaks were selected as the basic experimental material. Steaks 5/8 inch thick and 3 inches in diameter were prepared from major round muscles of choice grade beef and were oven-broiled 3 inches from the heating elements for 12 minutes on the first side and 10 minutes on the second. These conditions produced an internal temperature of about 75°C., which was sufficient for complete enzyme inactivation (*8*). This cooking schedule produced a medium well done steak. Either all steaks to be used in a given test series were prepared from the same carcass or steaks prepared from different carcasses were randomized with respect to treatment variables. All samples were prepared this way unless otherwise indicated.

Some tests were duplicated using "grillettes" cooked in the above manner. The grillettes were prepared by shredding lean choice grade beef rounds and pressing them in a mold to obtain patties 3 inches in diameter and 5/8 inch thick. Some 3/4-inch steak samples were also prepared by a "searing" plus 66°C., 53% relative humidity enzyme inactivation procedure for consumer acceptance and storage evaluations—a procedure reported to give superior consumer acceptance (*29*).

Organoleptic Evaluation. An expert panel of 10 persons was selected from an initial screening of 20 members of the laboratory staff and trained to differentiate between various levels of irradiation flavor intensity. Samples of beef irradiated with 0, 1.5, 3.0, and 4.5 megarads at ambient temperature were used for training this panel. Sufficient sessions were conducted to ensure that the panel could distinguish between various quantitative levels of irradiation flavor and could effectively duplicate its responses. The scale used in these experiments had the following numerical and verbal designations concerning amount of irradiation flavor:

1. None
2. Very little
3. Little
4. Moderate
5. Much
6. Very much

Panel sessions were conducted by warming the product to serving temperature in a double boiler, then serving four or less randomized samples to each panel member. Panel scores were normalized for each sitting by testing a control sample which had been irradiated at room temperature to a dose of 3.0 megarads. These samples usually were rated about 4.0 (moderate). The panel was tested at intervals on its ability to differentiate between samples receiving 0, 1.5, 3.0, and 4.5 megarads of irradiation at room temperature and performed satisfactorily.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch004

Consumer acceptance was evaluated by panels made up of 75 or more laboratory staff members who had had no recent experience with irradiated products. Panel members were not informed of the nature of the test. Each panelist received one irradiated and one nonirradiated control steak and was asked to rate each sample on over-all acceptability, using a 7-point Hedonic scale with 1.0 equal to "dislike very much," 4.0 "neither like nor dislike," and 7.0 "like very much." Half of the panel members received the irradiated product first, and half received the nonirradiated control first.

Sample Irradiations. All samples were irradiated under essentially isothermal conditions. Most of the samples were irradiated at Cook Electric Co.'s Inland Testing Laboratories cobalt-60 source at Morton Grove, Ill., using the temperature control system developed by the Quartermaster Food and Container Institute (*45*). The amino acid solutions and the samples used in the evaluation of carcass variation, grade, and cut by consumer acceptance panels were irradiated in the cobalt-60 source at the U. S. Army Natick Laboratories, Natick, Mass.

Process Variable Study

Initial Screening of Process Variables. A 2 × 5 factorial experiment was designed for the first test series, using a high and low extreme for each of five factors. Intermediate points of each factor were included to obtain a sense of direction.

The five variables and ranges selected for the initial studies were:

1. Irradiation dosage from 0 to 6.0 megarads.
2. Temperature of irradiation −25°, −80°, and −196°C.
3. Rate of cooling prior to irradiation

    Slow. Cooled to 4°C. in cooler, then to −20°C. in cold room, then to irradiation temperature in a vapor phase cooling box.

    Intermediate. Vapor phase cooling to irradiation temperature.

    Fast. Samples packed in cans and cans immersed directly in liquid nitrogen.
4. Rate of warming after irradiation

    Slow. In an insulated box initially at the irradiation temperature.

    Intermediate. In static room temperature air.

    Fast. Immersed in running water at about 15°C.
5. Irradiation environment: vaccum, nitrogen backfill, and oxygen backfill.

Statistical analysis of the data from this experiment showed that the only clearly significant variables were irradiation temperature and dosage. The over-all mean scores for temperature and dosage are tabulated in Table IV. Smaller but possibly significant effects of warming rate and packaging environment are shown in Figure 6, complicated by interaction with the irradiation temperature. The effects of both packaging and warming rate variables were largest at −196°C. The lowest irradiation flavor intensity

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scores (at 6-megarad dose) were obtained by irradiation in oxygen followed by fast warming.

The panel also examined samples irradiated at −196°C. using various warming and cooling rates for texture variation. Some samples fast-cooled to −196°C. were described as having a texture similar to "dry turkey

Table IV. Mean Irradiation Flavor Intensity Scores[a] of Enzyme-Inactivated Beefsteaks after Irradiation at 6.0 Megarads in Initial Process Variable Screening Experiment

Statistically Significant Variables	*Scores*
Temperature (over-all), °C.	
−25	3.32 (little +)
−196	2.22 (very little +)
Irradiation dosage (over-all), megarads	
6	3.01 (little)
3	2.52 (little −)
Irradiation dosage at −196°C., megarads	
6	2.48 (very little +)
3	1.65 (very little −)

Reference standard irradiated to 3.0 megarads at 20°C rated 4.0 (moderate).

[a] Based on 6-point scale, 1.0 = none to 6.0 = very much.

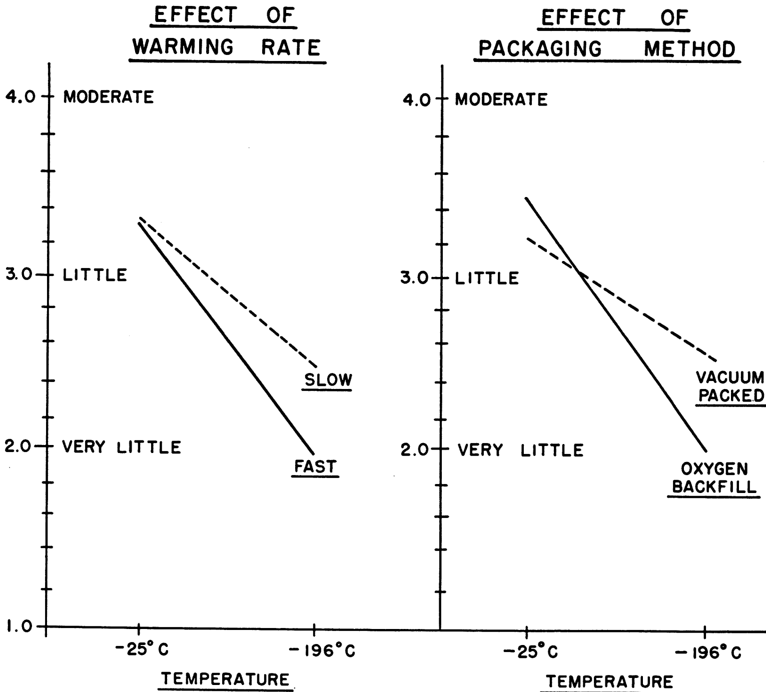

Figure 6. Possible significant interaction effects in initial 2 × 5 factorial process variable screening experiment

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breast," but texture differences were found to be too small and inconsistent to be measured by the panel.

The cooling rate showed no appreciable effect on flavor scores and was eliminated as a variable in subsequent tests. Since there was some indication of a tendency toward texture deterioration on rapid cooling, a standard method of cooling to 4°C. followed by vapor phase cooling (essentially slow) to irradiation temperature was followed in subsequent studies designed to measure the effects of the other variables more accurately.

Effect of Irradiation Temperature. A series of samples prepared from a single muscle was canned under vacuum and irradiated to 6.0 megarads at +25°, −25°, −80°, −140°, and −196°C. Because of the possible effect of packaging environment, a second series was prepared in an oxygen-backfilled pack. All samples were warmed to 4°C. in running water (fast warming) following irradiation, and each temperature-packaging condition variable was evaluated four times by the expert panel.

The irradiation flavor scores in Table V show quantitatively the dependence of irradiation flavor intensity on irradiation temperature for both oxygen-packed and vacuum-packed steaks brought to 4°C. rapidly after irradiation.

The relationship between irradiation flavor intensity and temperature is essentially linear for both packaging methods, except for the temperature of −140°C. where in both cases the irradiation flavor intensity observed was too high. This suggests that the temperature control system may have failed during the irradiations at this temperature. The flavor scores shown in Table V for a second set of samples (from a different animal) irradiated at −140° and −196°C. in a subsequent experiment support this suggestion.

Effect of Postirradiation Warming Rate. The effect of postirradiation warming rate was studied at −196°C. and 6.0 megarads for oxygen- and vacuum-packed steaks under conditions designed to maximize any possible effects. The postirradiation warming was carried out under the following conditions:

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Table V. Effect of Irradiation Temperature on Irradiation Flavor Intensity of Steaks Irradiated at 6.0 Megarads

	Irradiation flavor intensity scores[a]			
Irradiation	*O_2-Packed*		*Vacuum-Packed*	
Temp., °C.	*1st series*	*2nd series*	*1st series*	*2nd series*
+25	5.1		4.5	
−25	4.4		4.3	
−80	4.1		4.0	
−140	3.5	2.8	4.2	2.6
−196	2.4	2.2	2.8	2.3

[a] Based on 6-point scale, 1.0 = none to 6.0 = very much.
95% confidence interval ± 0.4.

FAST WARMING RATE. Eight cans of steaks packed in an oxygen atmosphere and eight cans of vacuum-packed steaks were immediately transferred from the irradiation cell to a flask containing liquid nitrogen and returned to our laboratories still at −196°C. These cans were immediately opened, and the steaks were transferred to plastic pouches and warmed to room temperature in a "radar" oven (approximately 1 minute in the radar oven was required). Total elapsed time from −196°C. irradiation to warming to room temperature averaged about 60 minutes. The actual warming from −196°C. to room temperature occurred in less than 5 minutes.

SLOW WARMING RATE. Eight cans of steaks packed in an oxygen atmosphere and eight cans of vacuum-packed steaks were held at −196°C. after irradiation for 96 hours and then allowed to warm slowly in an insulated box, warming from −196° to 4°C. in 31 hours.

The product from each of the four conditions (along with a standard) was rated for irradiated flavor by a panel of eight judges at each of four sittings.

The irradiation flavor scores from this experiment are shown in Table VI along with the 95% confidence intervals of the flavor scores.

Table VI. Effect of Postirradiation Warming Rate on Irradiation Flavor Scores[a] of Steaks Irradiated at −196°C. at 6.0 Megarads

	Postirradiation Warming Rate		*Analysis of Warming Rate Differences, D*	
Packaging Method	*Fast*	*Slow*	*D*	*90% confidence interval*
O_2 backfill	2.46 ± 0.37	2.93 ± 0.37	−0.47	±0.43
Vacuum-packed	2.57 ± 0.37	2.26 ± 0.37	+0.31	±0.43

[a] Based on 6-point scale, 1.0 = none to 6.0 = very much.

The factorial analysis of variance made on these data showed a large and highly significant packaging method–warming rate interaction but an essentially zero over-all warming rate effect owing to the opposed direction of the effect on oxygen- and vacuum-packed steaks. The separate analysis of differences *D* in Table VI indicates that the warming effects are probably real (but not at a 95% confidence level). Data from several additional test series (not reported in detail here) consistently indicated that slow warming rates reduced irradiation flavor intensity in vacuum-packed samples and increased it in oxygen-packed samples.

Differences owing to warming rate are also to be expected on the basis of electron spin resonance studies (*37*) of protein and peptide systems which indicate that the free radicals observed on −196°C. irradiation differ from those observed on room temperature irradiation and that the types observed at −196°C. are transformed to the form observed at room temperature on warming. Since some of the low temperature free radicals have half-lives of the order of days at −196°C., long holding times and/or slow

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warming rates would be needed to permit them to react before converting to high temperature radicals.

Since both the rates of low temperature radical reactions and rates of conversion of radicals from low to high temperature forms may be temperature-dependent, another experiment was performed to determine whether the rate of warming or the period of holding at low temperature (or both) was the important treatment variable.

Table VII. Effect of Postirradiation Heating Rate and Postirradiation Holding Time at −196°C. on Irradiation Flavor Intensity Scores[a] of Steaks Irradiated at 6.0 Megarads

	Rate of Postirradiation Warming		
Time Held at −196°C., Days	*Slow*	*Fast*	*Warming rate difference slow–fast*
0	3.04	3.25	−0.21
8	2.79	2.71	+0.08
Difference due to holding	0.25	0.54	

[a] Based on 6-point scale, 1.0 = none to 6.0 = very much.

Sixteen 300 × 200 cans of steaks were prepared from a single inside round of U. S. Choice beef. The steaks were vacuum-packed, using three cycles of nitrogen flushing. All cans received 6.0 megarads of gamma irradiation at −196°C. The mean irradiation flavor intensity scores under each of four processing conditions for the vacuum-packed steaks are shown in Table VII. Each sample was evaluated four times by an eight-member trained panel.

Statistical analysis shows that the difference owing to the warming rate alone is not significant at the 95% confidence level. However, the average difference owing to holding at −196°C. is significant at the 95% level by the one-sided t test. Some interaction is reflected in the data because the slow warming condition itself involves a period of several hours, during which the samples are held near −190°C.

Effect of Packaging Environment. Because of the opposing effects of postirradiation warming rate on steaks packed in an oxygen atmosphere and on vacuum-packed steaks discussed above, it was not possible to establish a clear preference on the basis of initial irradiation flavor intensity scores.

To determine the effect of packaging environment on the storage stability of steaks irradiated to 6.0 megarads at −196°C. and warmed slowly after irradiation, steaks were prepared in an oxygen atmosphere, in a nitrogen atmosphere, and in vacuum. The nitrogen series was included as an alternative method of obtaining an oxygen-free pack. After initial evaluation by an eight-member trained panel at two sittings, the −196°C.-irradiated samples were placed in 100°F. storage, and the controls were placed

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch004

in 4°C. storage. The panel again evaluated the samples after 1-month and 3½-months storage. The results of these evaluations are shown in Table VIII.

Statistical analysis showed no differences owing to time or treatment at the 95% confidence level. However, the scores for vacuum-packed steaks were significantly lower at the 90% confidence level, confirming previous data favoring vacuum packaging under slow warming conditions.

During the storage periods, steaks packed in an oxygen atmosphere developed a "metallic" or "bitter" off-flavor, and the steaks packed in a nitrogen atmosphere became dry and mealy in texture. Vacuum-packed steaks stood up best during the storage period.

Table VIII. Irradiation Flavor Intensity Scores (with 95% Confidence Intervals)[a] of Steaks Irradiated at −196°C. at 6.0 Megarads after Storage at 100°F.

Packaging Method	*Storage Period, Months*		
	0	*1*	*3½*
O_2	3.2 ± 0.6 (little)	2.7 ± 0.5 (very little +)	3.2 ± 0.5 (little)
N_2	2.6 ± 0.6 (very little +)	2.5 ± 0.5 (very little +)	2.9 ± 0.5 (little −)
Vacuum	2.8 ± 0.6 (little −)	1.9 ± 0.5 (very little)	2.7 ± 0.5 (very little +)

Sample irradiated at 3.0 megarads at room temperature rated 3.3±0.5 at the initial panel evaluation.

[a] Based on 6-point scale, 1.0 = none to 6.0 = very much.

Effect of Grade, Cut, and Animal Variations. Samples were prepared from the half loins (rib eye) and top rounds of two U. S. Choice and two U. S. Commercial grade beef carcasses. Half of each set of samples received 6.0 ± 1.5 megarads of irradiation at −196°C. at the Natick irradiation facility. The other half of the samples was frozen and held as unirradiated control.

These samples were evaluated by both the trained irradiation flavor intensity panel and consumer panels at the Swift & Co. Research and Development Center. No effects owing to grade or cut of meat were found, but statistically significant animal-to-animal variations were found by both panels.

Optimum Conditions for Preparations of Irradiation-Sterilized Steaks. The results of the process variable study reported above, along with our previous study (*31*) of steak preparation variables, lead us to recommend the processing conditions in Table IX for preparing shelf-stable beefsteaks of maximum acceptability. The processing variables having the greatest effects on irradiation flavor intensity and organoleptic acceptability are

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Table IX. Optimum Steak Processing Conditions

Sample should be lean and free of gristle.

Steaks at least ¾ inch thick should be surface-seared, enzyme-inactivated at 150°F. for 10 minutes in 50% relative humidity and then vacuum-canned.

Freeze-canned steaks using normal refrigeration procedures.

Irradiate to required dose at −196°C.

Transfer cold product to well-insulated storage area and bring to room temperature as slowly as possible.

Steaks should be reheated for serving in an oven broiler to the desired degree of doneness.

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irradiation dose and irradiation temperature. Evaluation of many samples irradiated at various temperatures to 3.0 or 6.0 megarads in our laboratory shows that irradiation flavor intensity scores generally fall within the limits shown in Figure 7 for each dose. The irradiation doses required for sterilization at lower temperatures have not yet been firmly established, but the 3-megarad and 6-megarad levels shown here represent probable lower and upper limits or the doses which will be required. Our experience indicates that steaks with irradiation flavor intensities higher than 3.0 on this scale probably will not be well accepted by consumers. On this basis, to make an acceptable product, an irradiation temperature of −196°C. is required for a 6-megarad dose while −80°C. is probably sufficiently low if a 3-megarad dose is used. We are currently attempting to determine more accurately the relationship between irradiation flavor intensity and consumer acceptance.

Consumer Acceptance of Irradiation-Sterilized Steaks. To determine the consumer reaction to irradiated steak produced under conditions shown

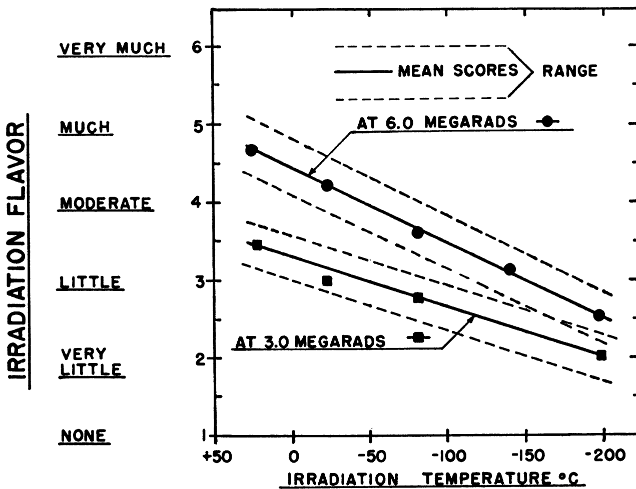

Figure 7. Expert panel irradiation intensity scores of beefsteaks as a function of temperature and dose

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by our trained panels to give the best product, vacuum-canned ¾-inch thick round steaks were prepared by searing at 700°C. and enzyme-inactivating at 66°C. and 53% relative humidity. Half of the samples were sterilized with 6.0 megarads of gamma irradiation followed by a 4-day hold at −196°C. The other half received the same thermal treatments but no irradiation. All samples came from the same round of U. S. Choice beef.

Mean acceptability scores and score distributions were almost identical for the irradiated and nonirradiated samples. The mean score for the irradiated sample was 4.36 and for the nonirradiated sample 4.38 (4.0 = neither like nor dislike and 5.0 = like slightly). Six panel members indicated no preference, 35 panelists preferred the irradiated sample, and 37 preferred the nonirradiated sample. Fifty-three percent of the panelists scored the two samples one point or less apart. No effect was observed on the relative scores owing to the order of sample presentation, although average scores for both samples were 0.7 point higher when the irradiation sample was presented first. The distribution of scores is shown in Table X.

The comments from the consumer panel indicated no particular single criticism other than that the nonirradiated sample was more frequently noted to be tough.

This test indicates that an acceptable irradiated steak product can be prepared and off-flavor caused by irradiation reduced to a point where other quality aspects of the steaks, such as meat quality, cookery, serving conditions, etc., are at least as important as extent of irradiation flavor in determining over-all consumer acceptance.

Table X. Responses of Consumer Panel to Irradiated (6 Megarads at −196°C.) and Nonirradiated Steaks Prepared Using Optimum Processing Conditions

Numerical Score	*Verbal Description*	*Number of Responses*	
		Irradiated	*Control*
7	Like very much	7	3
6	Like moderately	11	13
5	Like slightly	19	25
4	Neither like nor dislike	17	18
3	Dislike slightly	16	11
2	Dislike moderately	6	5
1	Dislike very much	2	3

Storage Tests. Steaks prepared using the processing conditions in Table IX were evaluated at intervals in a one-year room temperature (24°C.) storage test. One set of samples taken from a single U. S. Choice round was irradiated to 3.0 megarads at room temperature to serve as internal panel controls. These samples were held at 24°C. for 1 week and then placed in −20°C. storage.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch004

Test panel scores over the 12-month test period are shown in Table XI. Since the irradiation flavor scores of the product held at −20°C. should not change appreciably with time, it appears that the indicated fluctuations in flavor intensity are caused by the taste panel and not by differences in the samples. Some panel evaluation difficulties were experienced with these samples, owing to a variable amount of edge charring which occurred during preparation of the steaks. The texture of these steaks remained good during the test period.

Table XI. Irradiation Flavor Intensity Scores (with 95% Confidence Intervals)[a] of Irradiated Steaks Stored at 75°F.

Storage Time, Months	*3.0 Megarads at 25°C., Controls*[b]	*6.0 Megarads at −196°C., Samples*	*Difference*
0.5	2.3 ± 0.5	1.6 ± 0.5	0.7
1.7	2.8 ± 0.1	1.6 ± 0.1	1.2
2.8	2.7 ± 0.3	2.5 ± 0.3	0.2
3.8	3.3 ± 0.4	2.2 ± 0.4	1.1
6.8	2.5 ± 0.5	2.2 ± 0.5	0.3
9.8	3.2 ± 0.2	2.1 ± 0.2	1.1
12.0	3.9 ± 0.5	2.0 ± 0.5	1.9

[a] Based on 6-point scale, 1.0 = none to 6.0 = very much.
[b] Stored at −20°C.

Correlation of Chemical Yields in Model Systems with Irradiation Flavor Intensity. The observed effect of irradiation temperature on irradiation flavor intensity in beefsteak is shown in Figure 7. For 6-megarad irradiation the flavor intensity scores at −80°C. are roughly 75% of those at +20°C. and at −196°C. are approximately 50% of +20°C. scores. Comparing these percentage decreases at each temperature with the corresponding decreases in chemical yields reported in Table III shows no obvious correlation beyond the fact that both irradiation flavor scores and chemical yields from the peptides decrease with decreasing irradiation temperature.

Summary

Model system studies show that radiochemical yields of the major products of peptide radiolysis are drastically altered when irradiations are done at liquid nitrogen temperatures rather than at 20°C.

The data support evidence from electron spin resonance studies by Henriksen (*22, 23, 24, 25*) and Gordy (*16*) showing that the radicals present in irradiated proteins at −80° to −196°C. are different from those found on room temperature irradiation. They found that the free radicals produced at −196°C. are relatively stable at that temperature, but on warming to room temperature these free radicals both react with each other and

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch004

convert to "room temperature" free radicals, the total reactions and yields depending on the rate of warming.

Organoleptic studies on irradiated beefsteaks showed that irradiation flavor intensity is significantly lower at lower irradiation temperatures and depends on postirradiation warming rates.

Procedures are given which permit preparation of shelf-stable organoleptically acceptable sterile beefsteaks using low temperature irradiation.

While the chemical and organoleptic results cannot be directly correlated, it should be remembered that only a small fraction of the known chemical products were included in this investigation. Investigation of irradiation temperature effects on yields of other chemical products may well produce better correlation with flavor scores.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch004

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RECEIVED November 9, 1965. Research at Swift & Co. Research and Development Center in cooperation with U. S. Army Natick Laboratories under Contract DA-19-129-QM-2000. Views and conclusions are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch004

5

Radiation Chemistry of Polyamino Acids in Aqueous Solutions

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

The radiation chemistry of compounds related to the proteins—the amino acids and peptides—is reviewed briefly. Experiments with aqueous solutions of polyamino acids showed that a major effect of radiation was cleavage of the peptide linkages with formation of amide and carbonyl groups. Yields of amide were increased in oxygen. Inversion of L*-glutamate residues to* D*-glutamate and exchange of carbon-bound hydrogen atoms for tritons in the solvent were observed in poly-α,*L*-glutamic acid. Several new amino acid residues were detected in irradiated poly-α,*L*-glutamic acid, poly-*D,L*-alanine, and poly-*L*-proline. The pH of the solution during irradiation affects the physical and chemical changes induced in the polyamino acids.*

The effects of ionizing radiation on proteins have been widely studied because of their relevance to radiation biology and food irradiation. Generally, this work has dealt with three related aspects: effects on the physical properties of proteins, effects on the biological properties of active proteins, and radiation-induced chemical changes. Effects on nutritional and organoleptic properties are of special importance to food irradiation.

Ionizing radiation can act in two distinct ways on organic substances. In the absence of water, in condensed systems or in concentrated solution, the predominant effects occur directly on the organic molecule and produce electronic excitations or ionizations which may lead to chemical modification. In dilute solution (1% or less) the major effects are the result of reactions between the solute and reactive species produced by the radiolysis of water. These "indirect" effects are the subject of this article.

It is generally believed that there are three main reactive species produced by the action of ionizing radiation on water (*32*): the hydrated electron (e_{aq}), the hydrogen atom (H·) which is formed by the reaction of an electron with a proton (*8, 11*), and the hydroxyl radical (OH·). The yields of these from γ-rays, in molecules per 100 e.v. are e_{aq} = 2.85 (*7*) or 2.75 (*9*). OH· = 2.3 (*20*), and H· = 0·6 (*2*).

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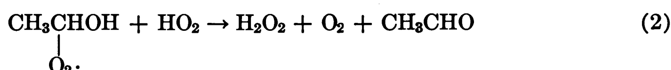
The electron is a reducing radical, and typical reactions are reduction of carbonyl compounds and metal ions. The hydrogen atom can act as a reducing agent, but more frequently in the kind of compounds considered here it acts as an oxidizing agent by abstracting another hydrogen atom:

$$RH + H\cdot \rightarrow R\cdot + H_2 \quad (1)$$

The electron is a more effective reducing agent than the hydrogen atom. The hydroxyl radical is an oxidizing species and in general also acts by abstracting a hydrogen atom. Kinetic studies of the reactions between hydroxyl radicals and amino acids (*30*) showed that the rate constants for glycine and alanine lie between 10^6 and 10^9 M^{-1} sec.$^{-1}$ and depend strongly on the charge state of the amino acid.

In oxygen, the electron and the hydrogen atom are both removed by rapid reactions to form the perhydroxyl radical (O_2^- and HO_2, respectively) (*8*).

Little is known about the reactions of the perhydroxyl radical, but like the hydrogen atom, it can act as either an oxidizing or a reducing agent (to give H_2O_2 and O_2, respectively). Its reactions are thought to be slower than those of the hydrogen atom and the hydroxyl radical (*40*). Weiss (*40*) has suggested a specific role for the hydroperoxy radical in reactions with organic peroxy radicals—e.g.,



$$CH_3\underset{\underset{O_2\cdot}{|}}{C}HOH + HO_2 \rightarrow H_2O_2 + O_2 + CH_3CHO \quad (2)$$

Interaction of the solute with radicals from the water is the first of a sequence of reactions which finally leads to stable products. Kinetic studies of the type cited give valuable information about the primary radical species and their relative reaction rates with molecules of different types. When sufficient data have been accumulated, it should be possible to predict the course of radiolysis in complex molecules. From the nature and yields of the products and by observing the effects on them of various factors such as concentration, pH, O_2, and specific radical scavengers, it is often possible to speculate about the mechanisms by which products are formed. More often than not, this is a difficult problem because the products, even from relatively simple compounds, prove to be complex. Furthermore, it is often possible to produce more than one mechanism to fit the experimental data. The proteins are particularly difficult because of their complex structures. They contain approximately 20 different amino acids with an average of more than three carbon atoms in the side chains, which vary considerably in their structure; hence, the possible number of products is large. For this reason, model compounds such as peptides and polyamino acids have been studied because they contain the peptide linkage but are free from the complications which arise from the diversity of the amino acid residues in a protein. A further practical difficulty which applies to chem-

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

ical studies is the severe treatment which is necessary to hydrolyze the protein for amino acid analyses. This is likely to alter the radiolysis products, and, therefore, the extensive studies of radiation-induced changes in free and substituted amino acids are an invaluable background for studies of proteins and protein models.

Radiation Chemistry of Amino Acids

Though the detailed radiation chemistry of the amino acids is beyond the scope of this article, it is worth making some general points which are relevant to the subsequent discussion on peptides and polyamino acids. Liebster and Kopoldova (*23*) give a comprehensive review.

First, the most frequently observed initial reaction appears to be the abstraction of a hydrogen atom. In the absence of O_2 this could be mediated by the hydrogen atom or the hydroxyl radical. In O_2 the hydroxyl radical is responsible.

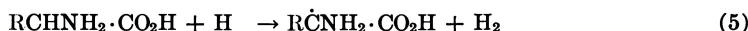
Reactions at the α-carbon atom of the α-amino acids can be divided into three classes.

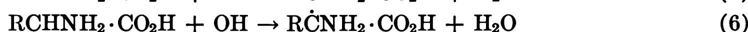
(1) Reductive deamination (*10, 37, 39*). This is accompanied by the formation of ammonia and a carboxylic acid. The mechanism proposed by Weeks and Garrison (*39*) for this reaction involves an initial removal of the amino group by H· :

$$RCHNH_2 \cdot CO_2H + H \rightarrow R \cdot \dot{C}H \cdot CO_2H + NH_3 \quad (3)$$

$$R\dot{C}HCO_2H + RCHNH_2CO_2H \rightarrow RCH_2 \cdot CO_2H + R\dot{C}NH_2CO_2H \quad (4)$$

(2) Oxidative deamination. This leads to the α-carbonyl derivative and is expected to proceed via an α-carbon free radical (*39*):


$$RCHNH_2 \cdot CO_2H + H \rightarrow R\dot{C}NH_2 \cdot CO_2H + H_2 \quad (5)$$


$$RCHNH_2 \cdot CO_2H + OH \rightarrow R\dot{C}NH_2 \cdot CO_2H + H_2O \quad (6)$$

$$2R\dot{C} \cdot NH_2 \cdot CO_2H \rightarrow RCH \cdot NH_2 \cdot CO_2H + RC{:}NH \cdot CO_2H \quad (7)$$

$$RC{:}NH \cdot CO_2H + H_2O \rightarrow RC{:}O \cdot CO_2H + NH_3 \quad (8)$$

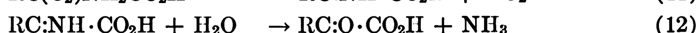
(3) Decarboxylation at the α-carbon. This leads to an amine by an unknown mechanism. This reaction is, to some extent, reversible (*24*).

Reactions at the α-carbon atom are affected by the presence of O_2. First, by rapidly removing the reducing species, it blocks reductive deamination. This is thought to explain the protective effect of O_2 on alanine destruction (*26*). Secondly, O_2 also probably alters the mechanism of oxidative deamination though the products (NH_3 and the carbonyl group) are the same (*39*).

$$RCH \cdot NH_2 \cdot CO_2H + OH \rightarrow R\dot{C} \cdot NH_2 \cdot CO_2H \quad (9)$$

$$R\dot{C} \cdot NH_2 \cdot CO_2H + O_2 \rightarrow RC(\dot{O}_2)NH_2 \cdot CO_2H \quad (10)$$

$$RC(\dot{O}_2)NH_2CO_2H \rightarrow RC{:}NH \cdot CO_2H + HO_2 \quad (11)$$


$$RC{:}NH \cdot CO_2H + H_2O \rightarrow RC{:}O \cdot CO_2H + NH_3 \quad (12)$$

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

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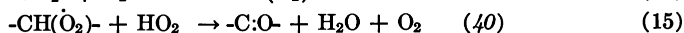
Yields vary with pH, and for the production of ammonia from glycine (*37*) and alanine (*33*) the point of lowest ammonia yield is the isoelectric point of the amino acid.

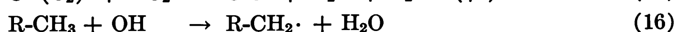
For glutamic acid (*18*) and glycine (*10*) the yield of ammonia varies approximately as the cube root of the concentration. This variation agrees with the "diffusion of the spur" model which derives from the hypothesis that at higher solute concentrations, water radicals are scavenged which would react with each other in more dilute solution. However, for the effect of cathode rays on the aromatic amino acids phenylalanine, tryptophan, and tyrosine and for cystine, this relationship is inverted, and amino acid destruction decreased with an increase in concentration (*29*).

Changes in aliphatic side chains are mainly initiated by the abstraction of a hydrogen atom from a C-H bond. This produces a free radical which in the absence of O_2 can combine with similar free radicals to give dimerization products or with CO_2 [probably the CO_2^- ion. (*40*)] to give monoaminodicarboxylic acids. In oxygen these are replaced by oxidative reactions which produce carbonyl, hydroxyl, and carboxyl groups through hydroperoxy intermediates:

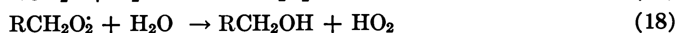
$$-CH_2- + OH \rightarrow -\dot{C}H- + H_2O \quad (13)$$

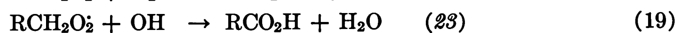
$$-\dot{C}H_2- + O_2 \rightarrow -CH(\dot{O}_2)- \quad (14)$$


$$-CH(\dot{O}_2)- + HO_2 \rightarrow -C{:}O- + H_2O + O_2 \quad (40) \quad (15)$$


$$R-CH_3 + OH \rightarrow R-CH_2\cdot + H_2O \quad (16)$$

$$RCH_2\cdot + O_2 \rightarrow RCH_2O_2\cdot \quad (17)$$


$$RCH_2O_2^{\cdot} + H_2O \rightarrow RCH_2OH + HO_2 \quad (18)$$


$$RCH_2O_2^{\cdot} + OH \rightarrow RCO_2H + H_2O \quad (23) \quad (19)$$

The proportion of reactions in the side chain, relative to those at the α-carbon atom, increases with the size of the side chain. In amino acids with a "reactive" side chain, such as the aromatic and sulfur-containing amino acids, this proportion is increased further. Similar trends are found in the data for amino acid destruction. Thus, Shimazu and Tappel (*34*) found that at pH 7 in $0.01M$ solution the most radiolabile among the amino acids studied were phenylalanine and methionine. They also showed an increase in radiolability with the size of the side chain for aliphatic amino acids. In comparing the radiolabilities of the amino acids, it should be borne in mind that pH and concentration effects vary from one amino acid to another.

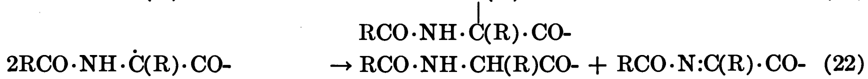
Radiation Chemistry of Peptides

Radiation-induced changes have been studied in a few water-soluble oligopeptides and substituted amino acids. Dale *et al.* (*10*) found a higher yield of ammonia from Gly-Gly than from glycine irradiated under similar conditions (x-rays, $0.13M$ solution, in vacuum) and also a large pH effect, the yield in HCl being less than one-third of that in neutral solution. The

ammonia yield from Gly-Gly-Gly was lower than from Gly-Gly. Liebster and Kopoldova (*23*) showed that recombination reactions were important in oxygen-free solutions (0.05*M*) of Leu-Gly-Gly irradiated with cobalt-60 γ-rays. After hydrolysis the following diaminodicarboxylic acids were found: diaminosuccinic acid (combination of two glycine moieties), dimethyldiaminoadipic acid (combination of glycine and leucine), and diaminodimethyl suberic acid (combination of two leucine moieties). Both of the products from leucine involve combination at C-5. Free amino acids were found in irradiated solutions of peptides by Hatano (*19*); thus glycine was formed from glycylleucine and glycyltyrosine and alanine from alanylvaline.

Garrison and Weeks (*16*) found that scission of the peptide linkage and cross-linking through the α-carbon atoms were the main reactions when solutions of acetylglycine or acetylalanine were irradiated in the absence of oxygen. The mechanisms suggested (Reactions 20–27) to account for the production of amide-like ammonia carbonyl groups and dimers all involved initial removal of the α-hydrogen atom.

$$\mathrm{RCO{\cdot}NH{\cdot}CH(R){\cdot}CO\text{-} + OH \rightarrow RCO{\cdot}NH{\cdot}\dot{C}(R){\cdot}CO\text{-} + H_2O} \qquad (20)$$



$$\mathrm{2RCO{\cdot}NH{\cdot}\dot{C}(R){\cdot}CO\text{-} \rightarrow \begin{array}{l} RCO{\cdot}NH{\cdot}C(R){\cdot}CO\text{-} \\ \quad\quad\quad\;\; | \\ RCO{\cdot}NH{\cdot}C(R){\cdot}CO\text{-} \end{array}} \qquad (21)$$

$$\mathrm{2RCO{\cdot}NH{\cdot}\dot{C}(R){\cdot}CO\text{-} \rightarrow RCO{\cdot}NH{\cdot}CH(R)CO\text{-} + RCO{\cdot}N{:}C(R){\cdot}CO\text{-}} \qquad (22)$$

$$\mathrm{RCON{:}C(R){\cdot}CO\text{-} + H_2O \rightarrow RCO{\cdot}NH_2 + O{:}C(R){\cdot}CO\text{-}} \qquad (23)$$

In the presence of O_2 Reaction 22 is thought to be replaced by:

$$\mathrm{\text{-}CO{\cdot}NH{\cdot}\dot{C}(R){\cdot}CO\text{-} + O_2 \rightarrow \text{-}CO{\cdot}NH{\cdot}C(\dot{O}_2)(R){\cdot}CO\text{-}} \qquad (24)$$

$$\mathrm{\text{-}CO{\cdot}NH{\cdot}C(\dot{O}_2)(R)CO\text{-} \rightarrow \text{-}CO{\cdot}N{:}C(R){\cdot}CO\text{-} + HO_2} \qquad (25)$$

$$\mathrm{\text{-}CO{\cdot}N{:}C(R){\cdot}CO{\cdot} + H_2O \rightarrow \text{-}CO{\cdot}NH_2 + CO(R){\cdot}CO\text{-}} \qquad (26)$$

or

$$\mathrm{\text{-}CO{\cdot}NH{\cdot}C(\dot{O}_2)(R){\cdot}CO\text{-} \rightarrow \text{-}CO{\cdot}NH_2 + O{:}C(R){\cdot}CO\text{-} + HO_2} \qquad (27)$$

Radiation Chemistry of Proteins

The radiation chemistry of proteins and other biological macromolecules has certain features which are not encountered with small molecules. Perhaps the most important difference concerns the dependence of the biological properties of the molecule on its secondary and tertiary structure. This structure is maintained by cystine bridges, electrostatic bonds, hydrogen bonds, and hydrophobic interactions. The energy required to break the noncovalent type of bonding is much lower than that required for a covalent bond, and it is possible that changes in secondary and tertiary structure are important effects of ionizing radiation on proteins in solution. The relative importance of secondary and covalent changes has been discussed by Augenstein (*4*) in a more general consideration of radiation-induced enzyme inactivation.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

In irradiated solutions of proteins, the reacting groups (the amino acid residues) are concentrated into small regions of the solution, and the water radicals are produced in small spurs (approximately 10 A. in radius). The kinetics of their interactions are therefore expected to be different from those of free amino acids which are distributed randomly throughout the solution. A brief treatment of this problem has been presented by Schwartz (*32*).

Furthermore, Mee, Navon, and Stein (*25*) have considered the possibility that reactive species such as the hydroxyl radical will attack groups on the outside of the protein in a nonspecific way, whereas less reactive species such as the hydrogen atom "may survive numerous collisions with different parts of the enzyme until they encounter more reactive parts—e.g., S-S bonds." Evidence for this was their finding that cystine S-S bonds of trypsin were especially vulnerable to attack by H atoms produced by electrodeless discharge from H_2 gas. There are other examples of differing radiolabilities of amino acids in proteins, irradiated in the solid state (*1*, *37*) and in solution; for example, Shimazu and Tappel (*34*) showed that cystine, methionine, phenylalanine, and histidine followed by threonine and tyrosine were the most radiolabile amino acids in 0.1% solutions of ovalbumin and catalase. Cystine, tyrosine, phenylalanine, proline, and histidine were found by Drake *et al.* (*13*) to be the most radiolabile amino acids in insulin (1% solution). This pattern is similar to that described for the amino acids, and it also seems likely that in general the nature of the products from the amino acid side chains will be the same in the proteins and the free amino acids. Reactions at the α-carbon atom are expected to resemble those found in peptide models—i.e., oxidative cleavage to carbonyl and amide groups and possibly some hydrolytic cleavage (*34*). Carbonyl and amide groups have been found in irradiated collagen (*5*), egg albumin (*31*), and pepsin and gelatin (*21*), and their absence was demonstrated in irradiated cytochrome *c* and hemoglobin (*3*).

The ratio of carbonyl to amide is usually much less than the 1:1 ratio expected from the mechanism given by Equations 20 to 27; the discrepancy could arise from limitations of the analytical method for carbonyls or from rapid removal of carbonyls by secondary radiation processes.

Radiation Chemistry of Water-Soluble Polyamino Acids

The synthetic polyamino acids are convenient models for chemical studies of proteins since they have the extended polypeptide structure of the proteins but are free from the complications which arise in the proteins from the large number of different side chains. The work described here has been confined to the water-soluble polyamino acids poly-D,L-alanine (PDLA), poly-α,L-glutamic acid (PGA), poly-α,D-glutamic acid, and poly-α,L-lysine, and the polyimino acid, poly-L-proline.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

Experimental

Materials and Methods. Polyamino acids were obtained from the YEDA Research and Development Co., Ltd., Rehovoth, Israel. Poly-L-proline was obtained in the water-soluble form (form II). Poly-α,L-lysine hydrobromide was converted to the phosphate by prolonged dialysis of a 1% solution against 1*M* KH_2PO_4 and then against three changes of distilled water. Other polyamino acids were used without further treatment. Amino acids were obtained from Koch-Light Laboratories, Ltd., Colnbrook, Bucks, England.

Tritiated water (200 mc./ml.) was obtained from the Radiochemical Centre, Amersham, Bucks.

For irradiation in vacuum, solutions were sealed in glass ampoules after thawing from the frozen state under 10^{-3}mm. of Hg. Irradiation under O_2 or N_2 was performed in a vessel which permitted continuous bubbling of the gas. The irradiation source was a 4000-curie cobalt-60 unit with a dose rate of 1 Mrad/hr.

Viscosities were measured in Ostwald-type viscometers in a water bath at 25° ± 0.01°C.

For automatic amino acid analysis (using a Technicon analyzer) samples were hydrolyzed in 5.6*N* HCl at 110°C. for 18 hours with rocking. Acid was removed by freeze-drying over NaOH pellets, and the residue was dissolved in 0.1*N* HCl. A sample containing 0.1–0.5 μmole of the amino acid to be measured was applied to the ion exchange column using a micrometer syringe. On adding acid, certain samples of PGA gave a precipitate, which would not dissolve during the heating period. In these cases a preliminary hydrolysis with pancreatin was used to break the polymer into acid-soluble fragments as follows.

A 50-mg. sample of pancreatin (British Drug Houses, Ltd., Poole, Dorset) was suspended in 2 ml. of 0.2*M* acetate buffer, pH 5.0, and insoluble material was removed. The PGA solution was adjusted to pH 5.0 by adding acetic acid or NaOH, mixed with 0.1 volume of the pancreatin solution, and incubated at 37°C. for 3 days in the presence of chloroform. Acid hydrolysis was then carried out as described for the irradiated polymer. Both D- and L-glutamic acids were measured by amino acid analyses as glutamic acid and γ-amino-*n*-butyric acid, respectively, after treating the samples at pH 5.0 with L-glutamate decarboxylase (*Escherichia coli*, Koch-Light Laboratories, Ltd., Colnbrook, Bucks). The enzyme was prepared by suspending the acetone-dried cells (10 mg. in 1 ml. of 0.2*M* acetate buffer, pH 5.0). The glutamic acid solutions (approximately 0.1%) in acetate buffer (1 ml.) were mixed with cell suspensions (0.1 ml.) and left overnight at room temperature. After acidifying with 0.5 ml. of 6*N* HCl, the precipitated protein was removed by centrifugation, and the supernatant solution made up to volume.

Total nitrogen estimations were performed on the polyamino acid solutions before hydrolysis by a semimicro-Kjeldahl technique. Amide-like nitrogen was measured on samples of the polyamino acids after hydrolysis in 2*N* HCl for 2 hours under reflux by the Conway microdiffusion technique using No. 2 units. Ammonia was absorbed in 0.5% boric acid solution and titrated with 0.01*N* HCl using a micrometer syringe.

Thin-layer chromatography of amino acids was carried out on layers of Kieselgel H (Anderman and Co., Ltd., London, S.E. 1), which were developed first in butan-1-ol acetic acid–water and dried in air overnight.

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After a second development, at 90° to the first in phenol-water (80 : 20) the plates were dried at 100°C. for 30 minutes, cooled, sprayed with a solution of ninhydrin in butanol (0.5% w./v.), and laid on a hot plate until the amino acids were just visible.

To measure tritium exchange, a solution of PGA (0.5%) in THO (1 mc./ml.) was irradiated under vacuum. THO was removed by freeze-drying; final traces of solvent and exchangeable tritium were then removed by dissolving the polymer in water (1 volume) and freeze-drying (repeated three times). The polymer was hydrolyzed as described previously and dissolved in water (1 volume). A refrigerated Packard Tri-Carb scintillation counter was used to count tritium in aqueous solutions (1 ml.) mixed with a dioxane-based scintillation fluid (20 ml.) containing PPO and POPOP. Counting efficiency, estimated by counting a sample of the solvent THO in the way described, was 10%.

Carbonyl compounds in irradiated PGA samples (0.1 ml. of a 1% solution) were measured by the method of Lappin and Clark (*22*) using α-ketoglutaric acid as a standard. For qualitative analysis, the 2,4-dinitrophenylhydrazones were prepared by mixing the irradiated polyamino acid solution (0.5 ml., 10% w./v.) with 2 ml. of methanol saturated with 2,4-dinitrophenylhydrazine and a drop of concentrated HCl and refluxed for 30 minutes. The solvents were removed under vacuum, and the residue was extracted with ether (three times) and then dissolved in water (2 ml.). Concentrated HCl (2 ml.) was then added, and the solution was refluxed for 5 hours. HCl was removed by flash evaporation, and the residue was dissolved in water. The solution was extracted with ether, made alkaline with ammonia, and again extracted with ether. The final ether extract was concentrated by evaporation, and samples were applied to thin layers of crystalline cellulose. The layers were prepared by applying a slurry of crystalline cellulose (Whatman CC41, Koch-Light Laboratories, Ltd., Colnbrook, Bucks) in 0.2*M* phosphate buffer, pH 7.4, and dried at room temperature. The developing solvent was *tert*-amyl alcohol, saturated with the same phosphate buffer.

Gel filtration of PGA samples (0.1 ml. of 1% w./v.) was performed on columns (0.9 $\times$ 120 cm.) of Sephadex G 75 (Pharmacia, Uppsala, Sweden) eluted with 2*M* NaCl. Fractions (2 ml.) were collected automatically, and the polymers were detected by measuring the absorbance at 230 mμ.

Enzymic hydrolysis of PGA by crude extracts of papain and pancreatin was carried out as described by Miller (*27*). Samples withdrawn at intervals were analyzed for free amino groups using a ninhydrin reaction (*41*). Values were corrected for the ninhydrin reaction of an enzymic digest which contained no PGA.

G values for the destruction of amino acids and formation of amide-like nitrogen were calculated from the initial slopes of linear plots of yield–dose data. For doses up to 1 Mrad in 0.1% solution, these were straight lines.

Reactions at α-Carbon Atom

Optical Inversion of Glutamate Residues in Poly-α,L-glutamic Acid. D-Glutamic acid has been detected in samples of poly-α,L-glutamic acid (PGA) irradiated in 1% solution at pH 6.8 (Na salt). The D-glutamic acid was measured in hydrolyzates of the polymer by an enzymic method (*36*). A specific enzyme, L-glutamate decarboxylase (*E. coli*), converts L-glutamic

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

acid to γ-aminobutyric acid, leaving any D-isomer unchanged. The latter two compounds can be separated and measured by automatic amino acid analysis. Table I shows the formation of D-glutamic acid in the dose range 0–40 Mrad (cobalt-60 γ-rays). By a similar technique it was found that L-glutamic acid was formed by irradiation of poly-α,D-glutamic acid.

Table I. D-, L- and Total Glutamic Acid in Poly-α, L-glutamic Acid Irradiated in 1% Solution at pH 6.8 (36)

(Amounts of D-, L-, and total glutamic acid expressed as moles/100 gram-atoms of total N)

Dose, Mrads	*L-Glutamic Acid, a*	*D-Glutamic Acid, b*	*Total Glutamic Acid*	*100b/(a + b)*
0	96.7	1.0	97.7	1.0
10	80.5	10.4	90.9	11.4
20	50.5	15.9	66.4	24.0
40	28.4	13.6	42.0	32.4

Detection of D-glutamate residues in irradiated PGA did not prove unequivocally that inversion was a result of irradiation; it could have occurred during the subsequent acid hydrolysis. However, when PGA was irradiated in tritiated water (THO), tritium was exchanged between the solvent and hydrogen atoms in carbon-hydrogen bonds of the PGA (Figure 1). The broken line in Figure 1 shows that the hydrogen exchange calculated from the isotope exchange is approximately 30% lower than expected from the inversion data. The difference is possibly caused by different reaction rates for the isotopes of hydrogen; such differences are known for the acid-catalyzed hydration of olefins by the three isotopes of hydrogen, and for tritium the ratio of abundance of T in the solvent to that in the product is 7.1 (*17*).

The extent of inversion ($G = 0.45$) is too high to be accounted for by the direct action of radiation energy at the α-carbon atom. Furthermore, the yield of D-glutamic acid was found to vary only slowly with concentration over the range 0.1 to 1% (w./v.), which implies an indirect action. It is most likely, therefore, that the first step in the inversion reaction is removal of the α-hydrogen by a hydroxyl radical or hydrogen atom (Reaction 20) to give a free radical on the α-carbon atom. Since this radical would be planar, recombination to glutamic acid could give either enantiomorph. Hydrogen atoms appear to have a specific reactivity towards the disulfide linkages and tryptophan residues or trypsin (*25*), which suggests that they have a relatively long lifetime in the absence of these groups. It is possible therefore, that recombination of the α-carbon free radical occurs directly with a hydrogen atom. Alternatively, the new hydrogen atom may be abstracted from another position in the polymer molecule, and such a possibility is implicit in the mechanism for peptide scission proposed by Weeks and Garrison (*39*) (*see* Reaction 22). The observed tritium exchange does

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

not distinguish between these possibilities. With the first possibility, tritium atoms would be formed by radiolysis of THO. In the second, tritium would rapidly exchange with the -NH- groups in PGA, and it is this hydrogen atom which combines with the α-carbon free radical in the mechanism represented by Reaction 22.

D-Glutamic acid was not found in irradiated solutions of free L-glutamic acid, which agrees with the results of Sharpless *et al.* (*33*), who found no D-alanine in irradiated L-alanine. However, Evans (*14*) found that a major loss of L-leucine-4,5-T, methionine-T(*G*), and phenylalanine-4-T amino acids stored in aqueous solution occurs as a result of racemization. In these cases, it seems likely that the inversion is a result of indirect action brought about by β-radiation from tritium decay.

Radiolysis of Peptide Linkage

When PGA or poly-α,L-lysine was irradiated with cobalt-60 γ-rays in neutral 1% aqueous solution in vacuum, the viscosity fell rapidly with dose (Figure 2). This effect suggests that the molecular weight of the polymer is decreased, and confirmation was obtained for PGA by gel filtration on Sephadex G75. Whereas the unirradiated polymer (average molecular

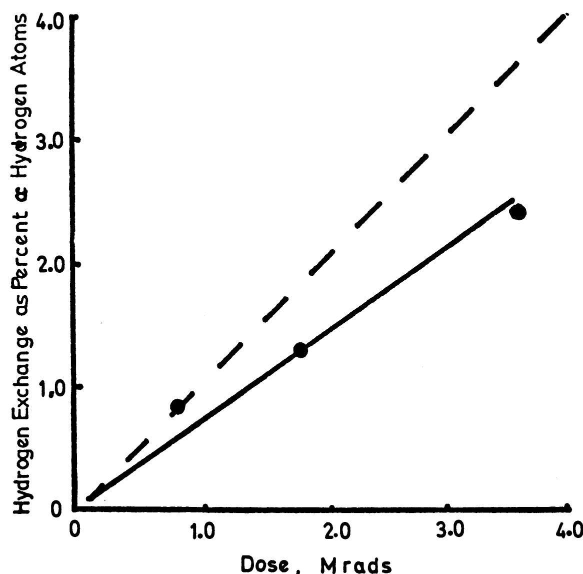

Figure 1. Radiation-induced hydrogen exchange in poly-α, L-glutamic acid

——— *Calculated from tritium exchange after irradiation in tritiated water (0.5% PGA in water containing 1 mc./ml. THO)*

— — — *Calculated from D-glutamic acid content after irradiation in 1% aqueous solution at pH 6.8 in vacuum*

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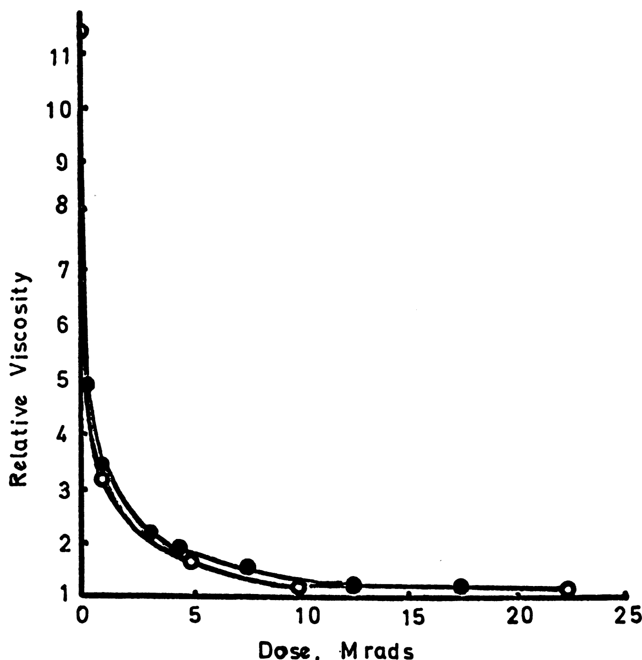

Figure 2. Change in relative viscosity of poly-α,L-glutamic acid (●) and poly-α,L-lysine (○) irradiated in 1% aqueous solution in absence of O_2

Viscosities measured at 25° ± 0.01°C. in Ostwald viscometer

weight = 90,000) was completely excluded by the gel, that which had received a dose of 1.0 Mrad in 1% solution contained fragments which were partially retained by the gel. The fraction of material retained by the gel increased with dose, and after 20 Mrads none was completely excluded. The average molecular weight of a 40-Mrad sample was low enough to be determined accurately by vapor pressure osmometry, and the value given by this method was 1300. A reduction in average molecular weight of this magnitude was most likely caused by scission of the peptide chain, and it had been shown (*39*) that the radiolysis of the peptide linkage in aqueous solution led to the formation of amide and carbonyl groups. Measurement of these groups in PGA irradiated under the conditions stated above showed that they were formed from PGA in approximately equivalent amounts (G amide =0.61, G carbonyl = 0.50), as expected from the mechanism of Weeks and Garrison (*see* Reactions 20–22). Furthermore, the carbonyl compound expected from PGA according to this mechanism is α-ketoglutaric acid, and this was identified as the only major component by thin-layer chromatography of the 2,4-dinitrophenylhydrazones formed by treating a 40-Mrad sample with 2,4-dinitrophenylhydrazine and hydrolyzing the product with acid.

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Assuming that amide formation represents the only way in which new chain ends are formed, it is possible to calculate an average molecular weight from the amide content $\left(\text{average molecular weight} = \frac{\text{weight of polymer}}{\text{no. of chain ends}}\right)$. For a 40-Mrad sample this gives an average molecular weight of 800. This value is slightly lower than that given by vapor pressure osmometry, which indicates that cross-linking, if it occurs at all, can be only a minor reaction.

Amide-like nitrogen was also formed when PDLA was irradiated in dilute (0.1%) aqueous solution; the yield ($G = 0.66$) was slightly higher than the yield from PGA. The yield of amide-like nitrogen from poly-L-proline was quite low ($G = 0.14$), as expected, since in this polymer the nitrogen atoms are held by three covalent bonds to carbon. It is possible that a secondary process is responsible for this reaction, though the proportionality of the yield of amide to dose in the range of 0.5 to 5 Mrads in 0.1% solution argues against this.

Effects of O_2 on Reactions at α-Carbon Atom

The yield of D-glutamic acid from poly-α,L-glutamic acid was very low in the presence of O_2 (1 atm.). This can be accounted for by either of the mechanisms discussed above since O_2 can combine both with H atoms and with the free radicals formed at the α-carbon atom (*see* Reactions 20–27).

The yield of amide-like nitrogen in PGA was increased from $G = 0.61$ to $G = 2.3$, in PDLA from $G = 0.66$ to $G = 2.0$, and in poly-L-proline from $G = 0.14$ to $G = 0.25$. This effect of O_2 is similar to that observed for the release of free NH_3 from the amino acids, though the increase for PGA and PDLA is greater than for the amino acids (*23*). The value for PGA in O_2 agrees closely with that reported by Sokol *et al.* (*35*).

The increase in amide-like ammonia formation can be explained by the mechanisms given in Reactions 20–27. From these it would be expected that the yield of amide-like nitrogen would be doubled by the presence of oxygen since in the absence of oxygen (Reactions 20–23) two α-carbon free radicals are needed to form one amide group. In the presence of O_2, every α-carbon free radical which combines with O_2 is transformed into an amide group (Reactions 20 and 24–27). It is also possible that in the presence of O_2, amide-like ammonia arises from reactions in the side chains. One such possibility for PGA is discussed in the next section.

Destruction of Amino Acids

Reactions in the Side Chain and Formation of New Amino Acids. Automatic amino acid analysis of polyamino acids irradiated in 0.1% oxygen-free aqueous solution showed that for PGA, PDLA, and poly-L-proline, amino acid destruction increased linearly with dose up to 1 Mrad. The G-values from these analyses were $G(\text{-Glu}) = 0.85$, $G(\text{-Ala}) = 1.9$, $G(\text{-Pro}) = 1.8$.

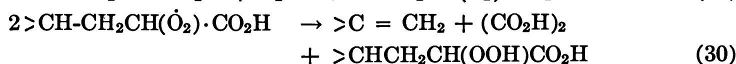
Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

For poly-α,L-glutamic acid, the amino acid destruction was mainly accounted for by the formation of amide groups ($G = 0.61$). The remaining nitrogen (0.24 mole/100 e.v.) was accounted for by new amino acids, about 19 in number, which were seen on thin-layer chromatograms and in automatic amino acid analyses. Most of these were formed in low yield ($G < 0.02$); among them were aspartic acid, threonine, serine, and alanine. Glycine was formed with $G = 0.1$, and this yield varied only slightly over the concentration range 0.1–1% (w./v.) of PGA, suggesting an indirect mechanism for its formation. Its identity was confirmed by two-dimensional TLC. Glycine has been found as a product of lysine ($G = 1.8$), irradiated with x-rays (*28*), and as the yield from lysine increased in the presence of O_2, it is likely that in this case it was formed by carboxylation of a CH_2NH_2 radical from the side chain. This sort of reaction is difficult to envisage for PGA, and in this case glycine was probably formed by removal of a glutamate side chain. Small amounts of glycine were detected by thin-layer chromatography in irradiated glutamic acid.

Oxygen increased the destruction of glutamate residues ($G = -3.5$), most of the increase arising from the increase in peptide-bond destruction described above. An unusual reaction of PGA in O_2 described by Sokol *et al.* (*35*) might account for a major part of the enhanced destruction. They found pyruvic acid to be a major product ($G \simeq 1$) when 0.15% solutions of PGA at neutral pH's were irradiated under 1 atm. O_2. The mechanism proposed for the formation of pyruvic acid was based on a bimolecular disproportionation of two γ-peroxy radicals.

$$>CHCH_2CH_2CO_2H + OH \rightarrow >CHCH_2\dot{C}H\cdot CO_2H \qquad (28)$$

$$>CHCH_2\dot{C}H\cdot CO_2H + O_2 \rightarrow >CHCH_2CH(\dot{O}_2)\cdot CO_2H \qquad (29)$$



$$2>CH\text{-}CH_2CH(\dot{O}_2)\cdot CO_2H \rightarrow >C = CH_2 + (CO_2H)_2 + >CHCH_2CH(OOH)CO_2H \qquad (30)$$

where $>CHCH_2CH_2CO_2H$ represents a glutamic acid side chain. The product $>C = CH_2$ then becomes the tautomeric form of a dehydropeptide, which on hydrolysis yields ammonia and pyruvic acid.

The perhydroxy radical formed on the γ-carbon atom (Reactions 30 and 31) is a likely precursor of aspartic acid, which we have found in yields of $G = 1.1$ when PGA was irradiated in 0.1% solution in O_2. Further oxidation and a decarboxylation step would be required to give aspartic acid. However, it is not yet known whether the aspartic acid is formed solely as a result of irradiation; it may be formed from a labile precursor during acid hydrolysis of the polymer. Our results differ from those reported by Friedberg and Hayden, who found high yields of aspartic acid in PGA irradiated in the absence of O_2; we found only traces of aspartic acid from solutions of PGA irradiated under N_2 (Figure 3). Glycine formation was not affected by the presence of O_2.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

Only three new amino acids were found (by automatic amino acid analysis) in poly-D,L-alanine after irradiation in 0.1% solution in the absence of O_2 with doses to 5 Mrads. All were in yields less than $G = 0.02$. The first was eluted before aspartic acid and was therefore acidic; the second was eluted in the position of aspartic acid, and the third in the position of glycine. We have not been able to confirm the identities of these products by TLC because of the low yields. The products found by amino acid analysis could not account for the discrepancy between amide-like ammonia formed ($G = 0.66$) and alanine destroyed ($G = 1.9$). Aspartic acid is formed when alanine is irradiated in solution (*26*), and it is likely that the carboxylation reaction proposed by these authors also accounts for the observed "aspartic acid" formation in PDLA.

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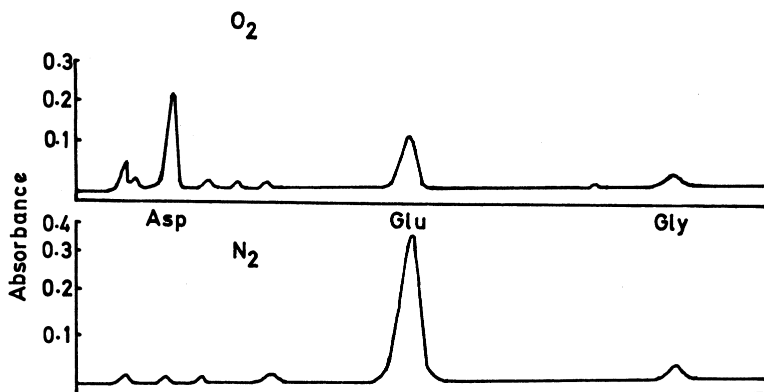

Figure 3. Portions of automatic amino acid analyses of PGA irradiated in 0.15% aqueous solution

pH 6.8, 4.5 Mrads, with O_2 or N_2 bubbled continuously

The presence of O_2 during irradiation of PDLA (0.1% solution, 1 atm. O_2), increased the amino acid destruction ($G =$ —1.9 in N_2 and 2.1 in O_2) and the formation of amide-like ammonia ($G = 0.66$ in N_2, 2.0 in O_2). The acidic amino acid formed in N_2 was not found in samples irradiated in O_2, but the amount of "aspartic acid" was increased. It is possible that O_2 converted the precursor of the unknown acidic amino acid into aspartic acid. The small amount of "glycine" apparently was not affected.

Friedberg and Hayden (*15*) reported that glutamic acid was formed when poly-L-proline was irradiated in dilute aqueous solution in the absence of O_2. We also found glutamic acid formation ($G = 0.14$) under similar conditions and confirmed the identity of the glutamic acid by two-dimensional thin-layer chromatography. A possible intermediate in this reaction

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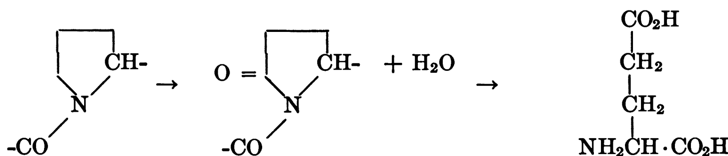
is the lactam of glutamic acid, which would be formed by the oxidation of the δ-carbon atom to a carbonyl group:



Glutamic acid was not formed when proline was irradiated under conditions similar to those described for poly-L-proline.

Effects of pH on Radiation-Induced Physical and Chemical Changes. When PGA or poly-α,L-lysine was irradiated in neutral 1% aqueous solution under vacuum, the viscosity of the solutions fell rapidly, indicating a preponderance of chain scission. Poly-L-proline and poly-D,L-alanine, irradiated under the same conditions, formed insoluble gels, indicating intermolecular cross-linking. It is found with the amino acids that the ability to form cross-links increases with the size of the side chain. However, the different effects for the polyamino acids cannot be caused by differences in the size of the side chain and appear to be connected with the charge on the polymer. Thus, cross-linking in PGA and poly-α,L-lysine was perhaps prevented by electrostatic repulsion of the charged molecules. This was borne out by the finding that the effect with PGA depended on pH. It was shown (Figure 4) that below pH 4, viscosity increased with dose, whereas

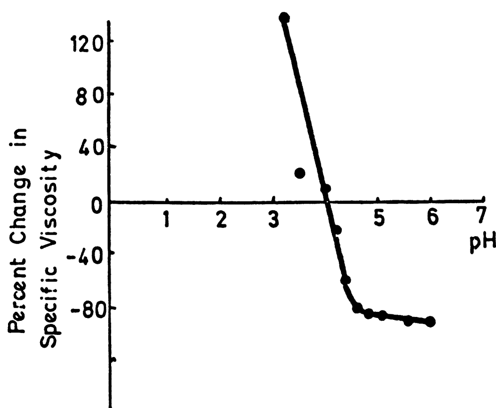


Figure 4. Effect of pH on radiation-induced viscosity change in 0.5% aqueous PGA (1 Mrad)

pH adjusted by addition of cation exchange resin. Readjusted to pH 9.0 with phosphate buffer for viscosity determination. Results expressed as percentage of viscosity of unirradiated PGA

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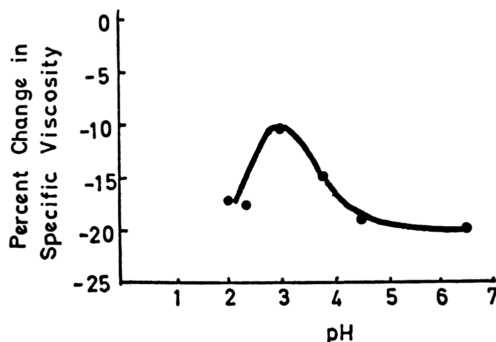

Figure 5. Effect of pH on radiation-induced viscosity changes in polylysine

0.5% solution of polylysine phosphate adjusted with phosphoric acid to required pH, irradiated in vacuo to 0.2 Mrad, pH readjusted to 5.0 with phosphate buffer for viscosity determination. Results expressed as percentage of viscosity of unirradiated polylysine

above pH 4 it decreased; at pH 4 there was no change in viscosity. The pH region in which this effect occurred coincides with the pK*a* of the glutamate carboxyl groups. It was expected that with poly-α,L-lysine, a similar effect would be found in the pH range 9–11, where the amino group loses its charge. However, the radiation-induced changes in viscosity remained unaffected in the pH 5–11 range, whereas there was a region in the acidic pH range in which a similar but less marked drop in viscosity occurred (Figure 5).

Viscosity changes in poly-D,L-alanine are difficult to interpret because of gel formation. In this case, the variation of the gel point (defined as the dose at which a 0.5% solution formed a stable gel) with pH was studied, and again it was found that the maximum degree of cross-linking (lowest gel point) was in the region of pH 4 (Figure 6).

Thus, in the three cases studied by viscosity change and gel formation, the effects of pH on radiation-induced changes occurred in the same pH region and were qualitatively similar.

If the number of radiation-induced cross-links exceeds the number of chain fractures, there is a net molecular weight increase; if the degree of cross-linking is sufficiently high, an insoluble network is formed. Charlesby (*6*) gives a detailed treatment of these processes.

The evidence is that for poly-D,L-alanine cross-linking is the predominant process; for poly-α,L-lysine chain fracture is predominant, and for poly-α,L-glutamic acid cross-linking predominates below pH 4 and chain fracture above pH 4. It is possible that under all conditions studied here all three polymers undergo partial cross-linking and chain scission; hence, pH could either suppress cross-linking at high pH or suppress chain fracture

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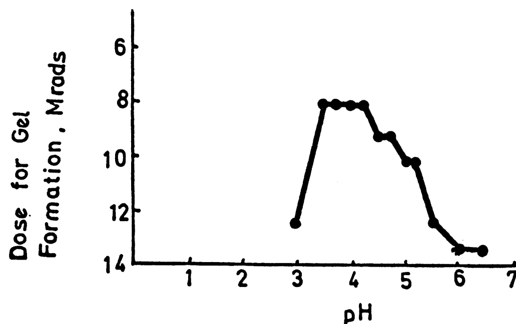

Figure 6. Effect of pH on radiation-induced gel formation in poly-D,L-alanine in absence of O_2
pH adjusted with NaOH and H_3PO_4

below pH 4. Furthermore, it is possible that the effects arise from changes in the relative proportion of inter- and intramolecular cross-linking. The available data are insufficient to decide between these possibilities. Evidence was presented above that PGA is degraded above pH 4.

With poly-α,L-glutamic acid, the more marked effect of pH on radiation-induced viscosity changes indicates that in this case there is probably an effect in addition to that found with poly-α,L-lysine and poly-D,L-alanine, and this probably arises from charge repulsion as discussed above.

In addition to the observed effects of pH on radiation-induced physical changes, striking effects of pH on the yields of certain products from PGA have been noted. In a detailed examination of the production of total carbonyls, labile ammonia, and α-ketoglutaric acid from PGA irradiated with cobalt-60 γ-rays under O_2 in 0.15% aqueous solution adjusted to pH values between 3.5 and 9.0 with NaOH or H_2SO_4, Sokol *et al.* (*35*) showed that the yields of labile ammonia and pyruvic acid depended strongly on pH, falling abruptly below pH 6. The yield of α-ketoglutaric acid, on the other hand, changed little over the entire pH range. Yields were independent of PGA concentration, showing that the effects were not caused by incomplete scavenging of OH radicals. Furthermore, similar experiments with the low molecular weight compound, N-acetylglutamic acid α-methyl ester, which showed no pH effects on yields, indicated that the effects with PGA were not caused by the change in charge on the side chain.

We have found that the yields of certain products from PGA irradiated in 1% aqueous solution under vacuum are reduced by altering the pH below 4. Thus the yield of D-glutamic acid decreased from $G = 0.45$ at pH above 5 to less than one-third of this value at pH 3.9; glycine ($G = 0.1$ at pH 7) could not be detected in samples irradiated at pH 3.9.

In the pH range 4–6, poly-α,L-glutamic acid undergoes a transformation from the α-helix, which is an uncharged, relatively rigid rod, to the random-coil form, in which the side chains are charged and the molecule is

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in continually changing conformation (*12*). Several factors could therefore contribute to the observed effects on product yields. There is no change in the yield of OH radicals in the pH range in question, so that the observations of Sokol *et al.* on PGA irradiated under O_2 are presumably caused by changes in the polymer. The mechanism for the formation of pyruvic acid and ammonia proposed by these authors (Reactions 28–30) requires an inter- or intramolecular interaction of two free radicals on the γ-carbon atom, and it was suggested that such interactions were more likely in the random-coil form of PGA which exists above pH 6 than in the α-helix, which is the form below pH 4.5. In the acidic range, it was suggested that the peroxy-free radicals were removed by reaction with a hydroperoxy radical (Reaction 31).

$$HO_2\cdot + \text{>}CH\cdot CH_2CH(\dot{O}_2)\cdot CO_2H \rightarrow O_2 + \text{>}CH\cdot CH_2CH(OOH)\cdot CO_2H \quad (31)$$

In the absence of oxygen, effects of pH on the polymer are complicated by possible effects of pH on yields of hydrogen atoms from the radiolysis of water. However, it is difficult to see how the increase in hydrogen atom yield at low pH could lead to a decrease in yield of D-glutamate and glycine. If these were formed by intermolecular reactions—e.g., Reaction 22 for forming D-glutamate—the explanation put forward by Sokol *et al.* to account for the effects in O_2 could also explain the observations on yields in the absence of O_2.

Conclusions

Knowledge of the radiation chemistry of proteins in solution is still scanty, despite considerable research. More information is required about the interactions of the primary radicals with the proteins, the nature of the products from individual amino acid residues, and the effects of such factors as concentration, protein conformation, O_2, and pH.

The work described here has shown that in dilute aqueous solution in the absence of O_2, a major effect of radiation on polyamino acids is scission of the peptide linkage with formation of amide and carbonyl groups. For PGA, this reaction more than accounts for the observed reduction in molecular weight.

Another major effect, found in PGA, is optical inversion of L-glutamate to D-glutamate residues. One implication of the radiation-induced optical inversion in proteins is that some modification of amino acids may pass undetected by the usual chemical analyses which do not distinguish between L- and D-isomers. Furthermore, introducing a D-amino acid residue into a protein could have a far-reaching effect on the secondary and tertiary structures, and this could have a more serious effect on the functional properties of the molecule than changes in the side chains. One "biological property" of PGA which is affected by irradiation in solution is its hydrolysis by proteolytic enzymes. The conformation of the polymer has a marked effect on its susceptibility to hydrolysis by certain enzymes (*27*), and we have

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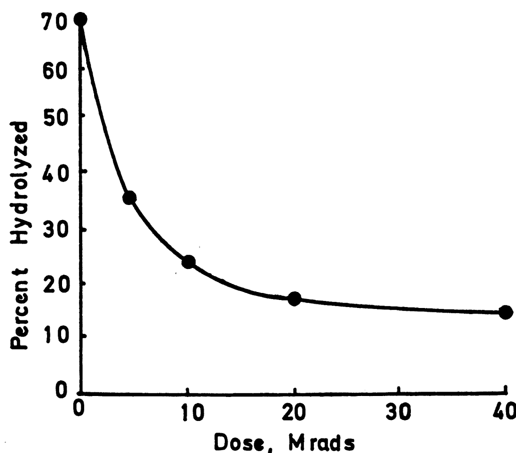

Figure 7. Effect of radiation on susceptibility of PGA to enzymic hydrolysis by pancreatin

PGA irradiated in 1% aqueous solution at pH 6.8. For enzymic hydrolysis, 0.2 ml. adjusted to pH 5.0, 0.1 ml. of enzyme solution (1.0 mg./ml. added), incubated at 37°C. for 24 hours. Extent of hydrolysis determined by ninhydrin reaction (41). Values corrected for blanks; (a) containing no PGA, (b) irradiated PGA with no enzyme

found that irradiation caused a rapid fall in the extent of hydrolysis by pancreatin and papain (Figure 7). It is likely that a large part of this effect arose from the introduction of D-glutamate residues.

The presence of O_2 causes an increase in the destruction of amino acids and in the amount of amide nitrogen formed and also influences the modification of amino acid side chains. Thus, a major product from PGA irradiated in O_2 is aspartic acid, which is formed only in traces in the absence of O_2. The inversion of glutamate residues is reduced in O_2.

Some unusual effects of pH on radiation-induced changes in amino acids were found. PGA showed a radiation-induced increase in viscosity below pH 4 and a decrease above pH 4. Poly-α,L-lysine showed a smaller viscosity decrease over the range pH 3–4 than at either side of this range. Poly-D,L-alanine showed a decrease in the dose required to gel a 0.5% solution in the pH range 3–4. Thus in all three cases, the effect of pH was qualitatively similar. An adequate explanation for these results is lacking except for PGA in which the charge on the side chains changes over the pH range in question and could allow cross-linking below pH 4 and prevent it above pH 4 by charge repulsion. Similarly, pH affects the product yields from PGA. In general, yields were lower below pH 4 than at higher pH.

Acknowledgment

The authors thank E. A. Bevis for his technical assistance, R. C. D. Jones for performing many of the amino acid and nitrogen analyses, J.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

Thomas for the molecular weight determination by vapor pressure osmometry, and A. J. Bailey for advice at various stages of the work.

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RECEIVED November 11, 1965. Work supported under Contract DA/91/591/EUC/2900, U. S. Department of the Army.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch005

6

Radiation Dosimetry in Relation to High Intensity Radiation Sources

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A dosimeter suitable for measuring the radiation intensity at doses in the range of 10^4 to 8×10^5 rads consists of a solution of 0.001M ferrous sulfate–0.01M cupric sulfate in 0.010N sulfuric acid. If the recommended concentrations are used, the dosimeter is reproducible to ±0.3% and stable after irradiation to approximately 2% per week. The dose received by the recommended dosimeter can be calculated, if read at 25°C., by converting the change in absorbance (ΔA) using the equation: dose (rads) = $\Delta A \times 6.43 \times 10^5$. This solution may be stabilized against thermal changes by preirradiating the solution to approximately 10^5 rads. This solution is then stable, changing approximately 2% per week.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch006

The Fricke dosimeter (ferrous sulfate solutions) has been used to measure the radiation intensity of various types of ionizing radiation sources since its development by Fricke and Morse in 1927 (*2*). It is widely accepted because it yields accurate and reproducible results with a minimum of care. This system meets many of the requirements specified for an ideal dosimeter (*5, 9*); however, it has a limited dose range, and for our applications it has been necessary to develop a dosimeter covering larger doses. Of the systems reviewed (*6, 7*), two (ferrous sulfate–cupric sulfate and ceric sulfate) showed the most promise for use with the radiation sources at the U. S. Army Natick Laboratories (*8*). Of these, the ferrous–cupric system has received the most use, and this paper describes our experience in using this system and suggests procedures by which it may be used by others with equal success.

The standard ferrous-cupric dosimeter as described in the literature (*3, 4*) consists of an air-saturated solution containing 0.001*M* $FeSO_4$, 0.010*M* $CuSO_4$, and 0.010*N* H_2SO_4. If prepared fresh daily, this solution is suitable for measuring doses in the range of 10^4 to 8.0×10^5 rads. Under controlled conditions, this system is reproducible to within 1%. A slight increase (±1%) in the uncertainty occurs as a result of batchwise variation. Com-

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parison of each new batch against a standard—e.g., a Fricke dosimeter—is, however, not required for reproducible results.

The above solution may be stabilized against thermal changes by preirradiating it to approximately 10^5 rads with ionizing radiation. This solution is then stable, changing approximately 2% per week.

The upper dose limit may be extended to more than 3×10^6 rads by increasing the ferrous ion concentration from $0.001M$ to $0.01M$. The range may be increased if the ferrous concentration is further increased (*9*).

Experimental Procedure

Ferrous sulfate–cupric sulfate solutions are prepared, varying the range of each between 0.0001 and $0.1M$ by dissolving $FeSO_4 \cdot 7H_2O$ (Baker analyzed), and $CuSO_4 \cdot 5H_2O$ (Fisher certified) in H_2SO_4 (Baker and Adamson) and diluting with distilled water (Barnstead single distilled).

Irradiations are carried out in Kimax glass ampoules. These ampoules are filled with 5 cc. of the solution, irradiated, using the apparatus previously described (*7*), and flame-sealed with a Perfe Keum Model HS-1 ampoule sealer. The irradiation source used for these experiments is a 1.3×10^6 curie cobalt-60 source consisting of two parallel plaques 56 inches wide by 48 inches high, spaced 16 inches apart. For most irradiations, the ampoules are placed in the center of a Masonite phantom which completely fills a No. 10 can (6 inches in diameter by 7 inches high). The can is placed in a fixed position in an aluminum carrier and transported into the irradiation cell to a predetermined position (*5*). The source is then elevated from the bottom of a 25-foot, water-filled pool into the irradiation position. After the desired exposure, the source is lowered to the bottom of the pool.

The time required to raise or lower the source is 1.5 minutes, comparable to an irradiation dose in the irradiation position of approximately 35,000 rads (transient dose). The dose rate in the irradiation position is approximately 60,000 rads per minute. Calibration of the ferrous-cupric dosimeter is determined by comparison with the Fricke dosimeter, when irradiated at a position in the cell having a lower dose rate. The dose rate in the calibration position is approximately 5×10^3 rads per minute with a transient dose of approximately 3×10^3 rads. Calibrations made at such a position when using a $G(Fe^{3+}) = 15.6$ for the Fricke dosimeter gave a $G(Fe^{3+})$ of 0.66 for the ferrous-copper dosimeter.

The ferric ion concentrations are determined by measuring the absorbance of the solutions, using a temperature-controlled (25°C.) spectrophometer with the absorption peak around 304 mμ. It is necessary to measure the extinction coefficient at the absorption peak for each instrument used. The molar extinction coefficients for our instruments are 2200 for the Cary model 15 at 302.5 mμ and 2352 for the Beckman model DB at 304 mμ.

Chemistry

It has been established (*3, 4*) that by adding cupric sulfate to modify the Fricke dosimeter, it is possible to reduce the $G(Fe^{3+})$ drastically from 15.6 to 0.66.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch006

The effect of the cupric sulfate is revealed by the following reactions (*1*):

$$Fe^{2+} + OH \rightarrow Fe^{3+} + OH^- \quad (1)$$

$$H + O_2 \rightarrow HO_2 \quad (2)$$

$$Cu^{2+} + H(e^-_{aq}) \rightarrow Cu^+ + H^+ \quad (3)$$

$$Cu^{2+} + HO_2 \rightarrow Cu^+ + H^+ + O_2 \quad (4)$$

$$Fe^{3+} + Cu^+ \rightarrow Fe^{2+} + Cu^{2+} \quad (5)$$

$$2Fe^{2+} + H_2O_2 \rightarrow 2Fe^{3+} + 2OH^- \quad (6)$$

Since oxygen is not consumed in the over-all reaction, it does not affect $G(Fe^{3+})$. This dosimeter shows no dose rate effect under the usual conditions of γ-ray irradiation since all of the above reactions except Reaction 6 are fast, and this reaction has a half-life of 14 seconds in $0.001M$ ferrous sulfate (*1*).

Experimental Results

When a system is being considered as a routine dosimeter, it is desirable to investigate the effect of altering various parameters—e.g., acid concentrations, reagent concentrations, and storage time—on the results obtained. To obtain this information, a series of experiments was conducted.

Effect of Acid Concentration. Figure 1 shows the effect of changing the acid concentration in a solution containing $0.01M$ copper sulfate and $0.001M$ ferrous sulfate. Minor variations in the acid concentration ($0.01N \pm 0.005N$) result in a variation of less than 5% in the G value. These

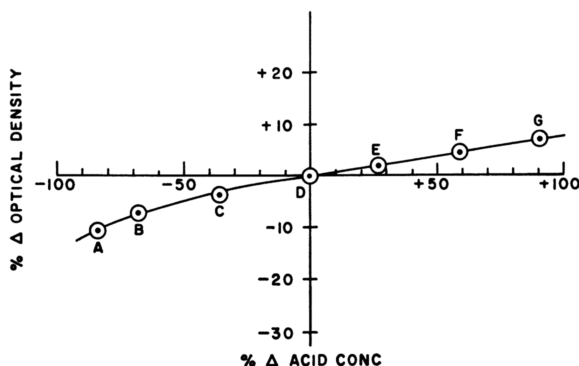

Figure 1. Effect of initial sulfuric acid concentration on ferric ion yield (ΔA), measured after irradiating of standard ferrous-cupric dosimeter to approximately 400,000 rads

$M 1.0.84N$ H_2SO_4 per 1000 ml.

A.	*2*	*E.*	*16*
B.	*4*	*F.*	*20*
C.	*8*	*G.*	*24*
D.	*12.6*		

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solutions were prepared by adding various volumes of 0.8N H_2SO_4 to preweighed salts of ferrous and cupric sulfate and then diluting to volumes with singly distilled water. Figure 2 shows the linear relationship of the change in absorbance of the irradiated solution as a function of irradiation exposure time (or radiation dose). Although the slopes of the curves for various solutions are similar, their apparent transient dose is different (35,000 and 76,000 rads). This could cause difficulties in using the system if the dose rate and transient doses are obtained by making a series of exposures for various times—e.g., 3, 5, 9, and 12 minutes—and then using the Y-intercept as the transient dose and the slope as the dose rate as described by Holm and Jarrett (*5*).

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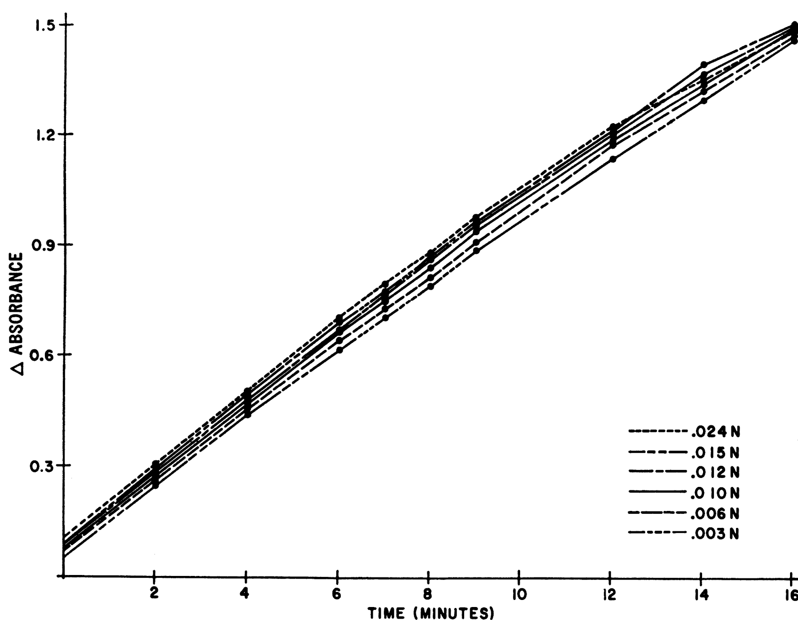

Figure 2. Effect of initial sulfric acid concentration on apparent transient dose, as determined by Y intercept

Effect of Ferrous Ion Concentration. The reactions involved in the ferrous-cupric dosimeter, as described by Hart (*1*), are independent of oxygen concentration, and one would not expect to observe a change in the ferric yield $G(Fe^{3+})$ in this system when increasing the dose, as occurs in the Fricke dosimeter. This would indicate that the dose limit of this dosimeter is a function of the initial ferrous ion concentration and is not influenced by the oxygen concentration. To explore this idea, we increased the ferrous ion concentration from 0.001M to 0.01M while keeping the

other components constant. As seen in Figure 3, the linear measuring range of this dosimeter is in fact increased beyond the 3-megarad limit of this experiment. This response has not as yet been explored completely and is the subject of a current study. The indications are that by increasing ferrous ion concentration and preirradiating the solutions, a usable system may be developed to compete with the ceric sulfate dosimeter in the range 10^4–10^8 rads. Obviously, when higher ferrous ion concentrations and higher doses are used, readings can no longer be obtained directly from spectrophotometer; the solution must be diluted with sulfuric acid before reading. This additional inconvenience can be more than compensated by the fact that the system may be considered as a secondary standard not requiring recalibration of each new batch of the solution against a standard such as the Fricke dosimeter, as is now required by the ceric system.

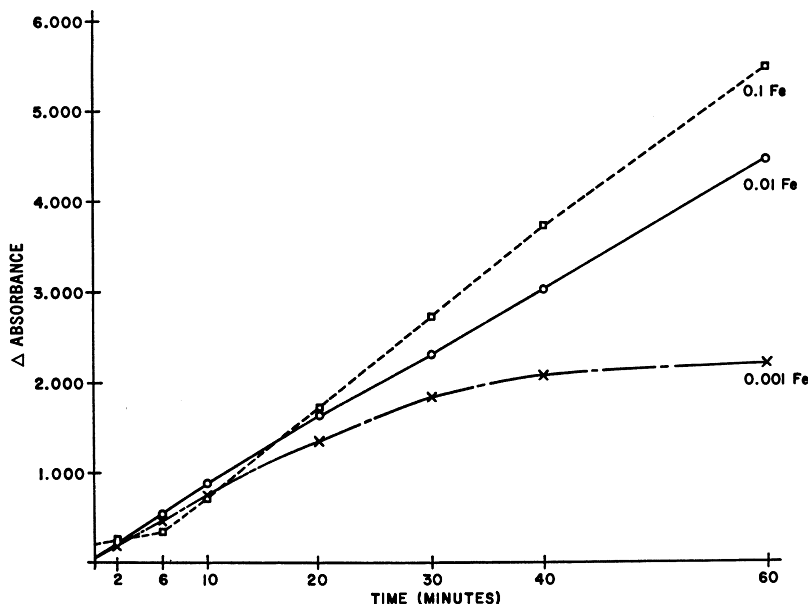

Figure 3. Effect of initial ferrous ion concentration on linear portion of absorbance–irradiation time (dose) relationship

Effect of Cupric Concentration. The cupric ion concentration was varied between $0.0001M$ and $0.1M$ to determine its effect on the "standard" ferrous copper dosimeter. Figure 4 shows that as the cupric ion concentration is increased, the $G(Fe^{3+})$ decreases. A change of $\pm 0.005M$ from an initial cupric ion concentration of $0.01M$ results in a $\pm 10\%$ variation in the ferric yield.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch006

Reproducibility. To determine the maximum variation in results that one might expect when using the ferrous sulfate–cupric sulfate system as a routine dosimeter, we prepared a number of dosimeters containing the standard solution and irradiated them in a No. 10 can similar to that previously described. These dosimeters gave a dose rate for the position of

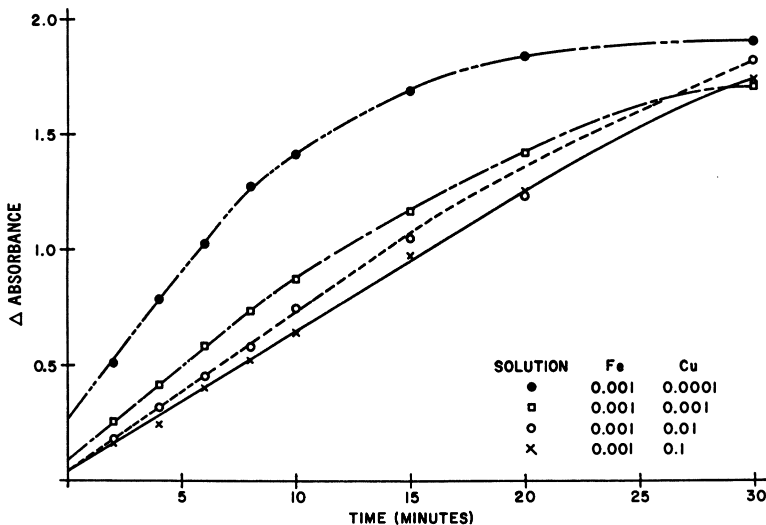

Figure 4. Relation of dose to absorbance curve of various initial cupric ion concentrations

$3.83 \pm 0.012 \times 10^5$ rads per minute or a reproducibility of $\pm 0.3\%$. Although this experiment was conducted under controlled conditions, variations between different solutions prepared by various experimenters did not exceed $\pm 1\%$. These solutions were prepared using reagent grade reagents and singly distilled water.

Storage Stability. The ferrous-cupric dosimeter has never received much attention as a dosimetry system because the unirradiated solution undergoes rather rapid changes during storage (Figure 5). To solve this problem, our first approach was to read the unirradiated (blank) solution against distilled water at the start of irradiation, then read all irradiated solutions against a distilled water blank rather than the unirradiated blank, and, finally, to subtract the two to obtain the change in absorbance. This procedure can be successful, provided the dose rate of the radiation source is relatively high or corrections are made for this autoxidation. Figure 6 shows that after the solution has been irradiated to an absorbance greater than 0.2, the change in absorbance with time is relatively stable (within 2% per week).

If the change in absorbance is made by comparing the irradiated with the unirradiated solution by using the unirradiated solution as the blank,

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the results will vary as a function of the time elapsed between the start of irradiation and the time at which the samples are read. This variation can lead to large errors. However, the stability of solutions irradiated in excess of 10^5 rads indicates that this provides a method of stabilizing the solution before use as a dosimeter. We have evaluated this technique for the standard ferrous–copper dosimeter and found it to be useful. Figure 7 shows the linearity of the change in absorbance *vs.* dose. This technique lowers the range of the dosimeter but provides a stable system. By increasing the ferrous ion concentration, it should be possible to overcome the disadvantage of lowered range.

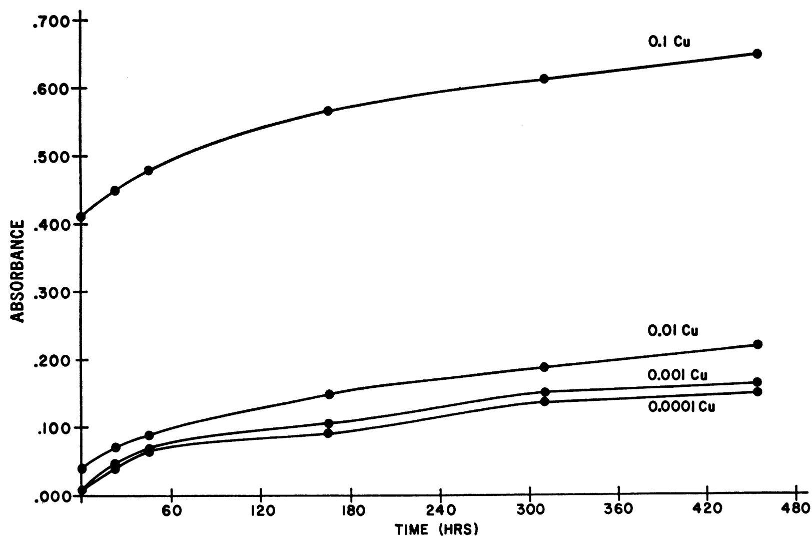

Figure 5. Storage stability of unirradiated ferric cupric solutions of varying cupric ion concentrations

Effect of Temperature during Reading. The molar extinction coefficient of Fe^{3+} was determined temperature-dependent in the temperature range 20°–55°C. to the extent of 0.59% per degree centigrade. It is, therefore, necessary to read the solutions in a temperature-controlled spectrophotometer or to correct for the temperature variation for 25°C.

Discussion

There is no question that the ferrous-cupric dosimeter can be used for routine dose measurements if the unirradiated solution is read at the start of the irradiation and not used as a blank against which the irradiated solutions are compared. The irradiated solutions can be held up to 1 month before reading without affecting the results by more than 10%. If it is inconvenient to prepare the solution fresh before each day of irradiation, the

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch006

old solutions can be used, provided a blank is read before the start of irradiation; alternatively, the solution can be stabilized by irradiating it so that it has an absorbance greater than 0.2 (approximately 10^5 rads). Preirradiation reduces the useful range of the standard dosimeter, but may not be a problem if the ferrous ion concentration is increased to 0.01*M*.

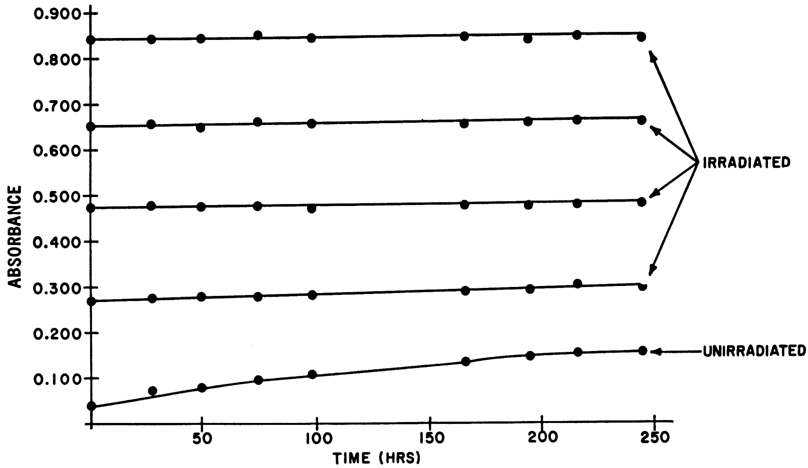

Figure 6. Storage stability of standard ferrous-cupric dosimeter, unirradiated and irradiated, to various initial absorbances
Ferric ion concentration

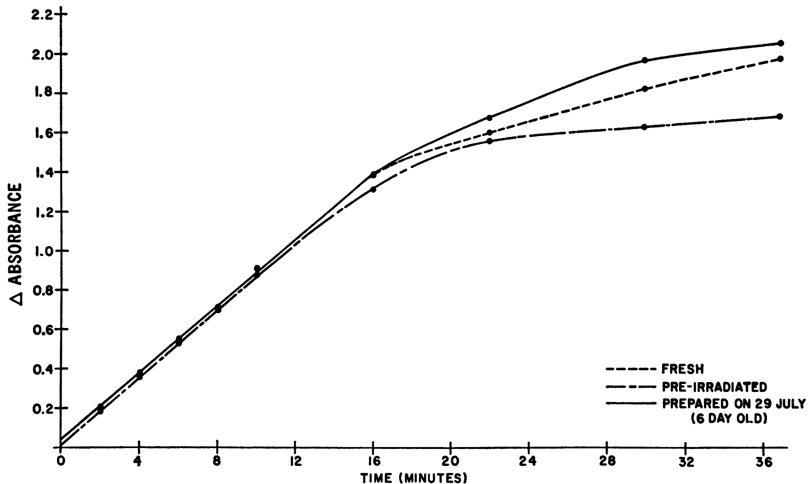

Figure 7. Relation of irradiation time to absorbance curves for fresh preirradiated and stored solutions of standard ferrous-cupric dosimeter

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch006

For routine use, the dose the standard ferrous-cupric dosimeter ($0.001M$ $FeSO_4$, $0.01M$ $CuSO_4$, in $0.01N$ H_2SO_4) has received can be calculated, if read at 25°C., from the following equation:

$$\text{Dose (rads)} = (A_i - A_u) \times 6.43 \times 10^5$$

where A_i = absorbance of irradiation solution

A_u = absorbance of unirradiated solution

6.43×10^5 = constant value assuming a G value of 0.66, molar extinction coefficient of 2200 at 25°C., density of 1.024, and unit conversion factors.

A correction of 0.59%/°C. must be made for the absorbances if the solution is read at a temperature other than 25°C.

Acknowledgment

The author expresses his appreciation to J. W. Halliday, R. M. Martin, N. W. Holm, and P. A. Alpert for their assistance in collecting the data reported in this paper.

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RECEIVED November 9, 1956.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch006

Radioactivity Criteria for Radiation Processing of Foods

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch007

The high-dose-level processing of foods with high energy electrons and x-rays can induce measurable radioactivity. Such radioactivity might, but fortunately need not, present a food wholesomeness problem. The amount of induced radioactivity can be controlled and limited so as to be nonmeasurable by limiting the maximum energy of the radiation to 10 m.e.v. Therefore, nonmeasurability is one useful criterion. Another criterion may be developed from considerations of maximum permissible concentrations of radionuclides in foods and of the deliberate or chance nature of the occurrence of the activity. The bases of these criteria and of possible future developments are detailed by considering characteristics of photonuclear reactions, detection methods and capabilities, influences of production parameters, and maximum permissible concentrations.

The general benefits derived from using radiation as an agent for processing materials have overshadowed and obscured detailed effects. This report examines one effect—the production of radioactivity in irradiated foods—that has been a deterrent to the participation of industry in the radiation processing of foods. Such a discussion and review of radioactivity production should help to identify the problem and should guide the establishment of future standards for processing irradiated foods for public consumption. This report reviews and emphasizes only certain aspects of radioactivity production and is not intended to be an exhaustive review of the subject.

The processing of all foods by high-energy radiation beams (electron, x-ray, or γ-ray beams) will produce excited nuclei of atoms that will emit

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In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

α-, β-, and/or γ-rays after the processing has been completed. In some cases, these radionuclides could linger in the irradiated foods for years and could represent potential health hazards if the levels of radioactivity were of any biological significance. All ionizing radiation is known to be carcinogenic, and therefore, the amount of radioactivity in foods intended for human consumption must be limited and controlled. Additionally, the radionuclides are regarded as food additives since the activities result from the radiation processing.

However, the research findings reviewed show that the maximum possible induced activity and its associated radiological effect resulting from electron beam processing, for example, are a fraction of the activity and effect resulting from those naturally occurring radionuclides such as potassium-40 and carbon-14, already present in foods. Furthermore, this activity, which can be reduced further under controlled conditions, is probably not significant radiologically; it is small compared with the established values of the maximum permissible concentrations of radioactivity in water. Indeed, the magnitude of the induced radioactivity is so small that it is difficult to measure except with the most refined detection equipment. Therefore, radioactivity produced in foods by radiation processing need not represent a public health problem if the conditions of irradiation are controlled.

Federal Controls on Radioactivity in Foods

Congress has enacted legislation, and the Executive Branch of the Federal Government has assigned responsibilities that apply to the problem of radioactivity in foods (*11*). In 1958, Congress enacted the Food Additives Amendment (*8*) of the Food, Drug, and Cosmetic Act (*3*), which states that "no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal." Congress, in defining "food additives" in this Act, expressly provided that the entire Food Additives Amendment does not apply to any substance which under the conditions of its intended use is generally recognized on the basis of scientific procedures as safe by competent scientific experts (or to substances used in food before January 1, 1958, on the basis of either scientific procedures or experience based on common use in food) (*19*).

The general control of radioactivity in foods has been provided by other congressional directives that have been executed by an Executive Order (*7*) delegating to the Department of Health, Education, and Welfare (HEW) the primary responsibility for all public health matters relating to radiation. This department, together with the Federal Radiation Council, recommends the federal policy for routine and emergency situations involving radiation. This policy is recommended to the President, the federal

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departments, and the state public health authorities for their guidance, adoption, and application.

HEW and the Federal Radiation Council have been assisted by the technical recommendations of the National Council on Radiation Protection and Measurements. The NCRP has been particularly involved in developing the concept and the values of the maximum permissible concentrations (MPC) of radionuclides in water, which values could be applied to foods (*16*). [The National Council on Radiation Protection and Measurements was created on July 14, 1964, by Public Law 88-376. The council is the successor to the unincorporated association which was known as the National Committee on Radiation Protection and Measurements and which for 35 years served as the focal point for developing radiation protection philosophy and standards in the United States. The current address of the NCRP is 4201 Connecticut Ave., N.W., Suite 402, Washington, D. C.]

Specific control of the added radioactivity induced during food processing has been provided through clearances issued by the Food and Drug Administration (FDA) for the production and release of irradiated foods to the public. The FDA issues a clearance when it is sure that the irradiated food is safe. A clearance is issued after the petitioner describes in detail the nature of his proposed process and furnishes data assuring the safe use of the processed food. To date several clearances have been issued or are pending for foods processed to sterilizing doses (5 megarads) when the radiation energies used are limited to values below 10 m.e.v. (*20, 22*). On the basis of the criterion employed by the FDA, it should be possible in the future to clear irradiated food processes using radiation energies higher than 10 m.e.v. if the dose magnitudes required are considerably less than 5 megarads.

The basis for the criterion developed and applied by the FDA has been the ability to measure the added radioactivity. The measurability of radioactivity is used as evidence for the presence of the radioactivity. Two sets of experiments have most extensively demonstrated the characteristics and the measurability of the radioactivity induced in foods. In the case of the work by the Stanford Research Institute (*9*), electron and x-ray beams with energies up to 24 m.e.v. were used. The results showed clearly defined but small activation in a number of important food elements. The data most relevant to foods were obtained by Meneely (*14*), who reported that "at and above 12 m.e.v., the isotopes Sc-47, Xe-133m, Mn-54, Sc-46, Na-24, and Sc-48 were detected and identified by energy and half-life measurements." However, when the electron beam energy applied to particular foods was less than 11.2 m.e.v., neither gamma- nor beta-emitting nuclides could be detected with the techniques used by Meneely, although the γ-ray scintillation spectrometric method used in his study was sufficiently sensitive to detect reliably the presence of induced gamma-emitting radionuclides in irradiated food at a level about one order of magnitude below

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the gamma-emitting radionuclides already present in the foods studied as they occur today in nature. Therefore, the lack of measurability in these experiments provides the criterion on the nonexistence of radioactivity production for radiation energies less than 10 m.e.v. and assures compliance with the Food Additive Amendment.

The measurability criterion used by the FDA is a reasonable, conservative, and compromise criterion because although radioactivity is known in principle to be produced at energies below 10 m.e.v., such radioactivity has not only been nonmeasurable in food samples but also is probably not radiologically significant. The criterion also simplifies control of the process considerably and eliminates the need for on-line monitoring of radioactivity induced by the process.

The only apprehension of prospective industrial radiation processors with the nonmeasurability criterion comes from the analogies with the history of pesticide residues in foods (*4*). Pesticides are cleared on the basis of either zero tolerance or finite tolerance, and use is rated as safe for a zero-tolerance pesticide when the residue on foods cannot be measured. However, improvements in measurement techniques with time have changed the definition of zero tolerance, sometimes abruptly. Radiation processors are apprehensive of similar experiences with radioactivity induced in food.

Characteristics of Radioactivity Resulting from Radiation Processing

Radiation beams induce radioactivity primarily by photonuclear reactions. In these reactions, the absorption of energy from the incident electron, x-ray, or γ-ray will produce an excited nucleus that will then emit a neutron, proton, triton, γ-ray, or other secondary radiation. The chart of the nuclides from carbon to sodium in Figure 1 demonstrates the type of nuclide resulting from the emission of a secondary radiation from a given parent nuclide (*10*). The threshold energies necessary for the incoming radiation to produce a secondary radiation are given for a few of the reactions most relevant to this report.

The photonuclear neutrons, protons, or tritons can be emitted almost immediately, whereas γ-rays can be emitted for time periods of the order of hours and days in what are called isomeric transitions. When a neutron, proton, or triton is emitted, the residual nucleus can in turn be radioactive and can emit positive or negative β-rays or γ-rays over long time periods. An additional important process is that in which the emitted neutron is reabsorbed in another nucleus which then becomes radioactive and emits β-rays or γ-rays. The two important steps in producing radioactivity are the absorption of the nuclear excitation energy and the re-emission of the energy in the form of nuclear radiations.

The probability for absorption of the nuclear excitation energy is determined by the threshold energy and by the probability magnitude at en-

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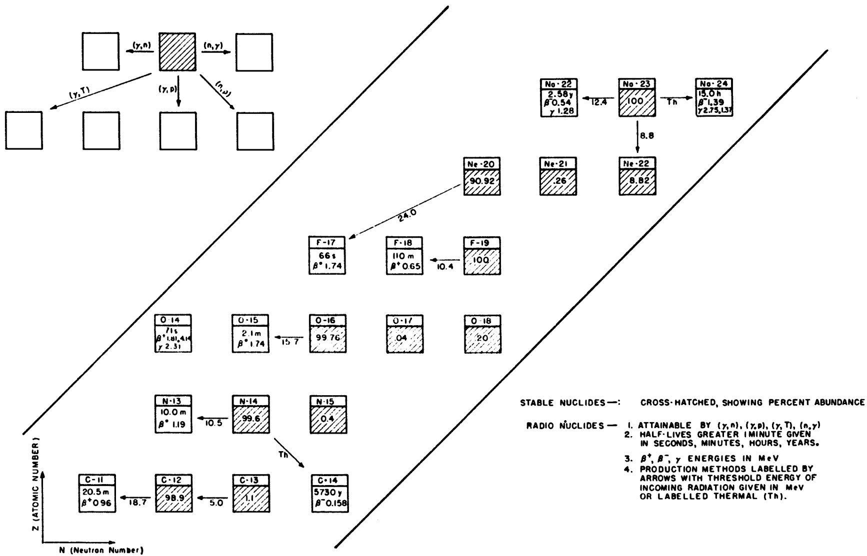

Figure 1. Chart of nuclides from carbon to sodium, illustrating various processes for production of radionuclides (10)

ergies above the threshold. The threshold energies vary with the element and with the process. A plot of threshold energies for photoneutron, photoproton, and phototriton reactions is given in Figure 2 as a function of the mass numbers (*2*). These data points show that the thresholds tend to decrease with increasing mass number with a few notable exceptions, one being the $^{13}C(\gamma,n)$ reaction with a threshold of 5 m.e.v. In addition, many photoneutron and photoproton reactions have thresholds below 10 m.e.v. Table I provides a specific listing of those nuclides with photoneutron, photoproton, or phototriton thresholds below 10 m.e.v., in order to emphasize the large number of these nuclides.

Fortunately, although the ratios of the photoproton to the photoneutron reaction probabilities can be as large as 4 for nuclides below ^{40}Ca in the periodic table, many photoproton reactions from the most abundant nuclides result in a final stable nuclide. However, since photoneutron threshold energies exist below 10 m.e.v., many nuclides can be made radioactive at energies below 10 m.e.v. This radioactivity could become measurable if the probabilities for the emission of a neutron, proton, triton, or γ-ray were large enough and if the processing radiation doses were high enough.

A number of investigators have examined the production of radioactivity resulting from photoneutron reactions induced by high-energy electrons. Meyer (*15*) has reviewed these data and showed that the induced radioactivity, R, in specific activity per unit dose (picocuries per gram of element times megarad) is reasonably well represented by Equation 1.

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$$R = \frac{4 \times 10^{-3}A}{T}(E - E_o)^3 \text{ in } \frac{\text{picocuries}}{\text{gram element} \times \text{megarad}} \quad (1)$$

where A = atomic number of target nuclide
T = half life of product activity in years
E = initial electron energy in m.e.v.
E_o = threshold energy for reaction producing product activity

The values given by Equation 1 for electrons are increased by about a factor of 10 for x-rays when the electron and x-ray energies are both about 30 m.e.v. This factor can be derived from data curves (*17*). As the radiation energies decrease towards the threshold energies, the x-ray-electron factor increases above the value of 10 at 30 m.e.v.

Another way of stating the conclusions of Equation 1 is to provide a plot of the induced radioactivity obtained experimentally by Glass and Smith (*9*) for electrons with energies of 24 m.e.v. The data plotted in Figure 3 show the general trend and magnitudes of the radioactivity in terms

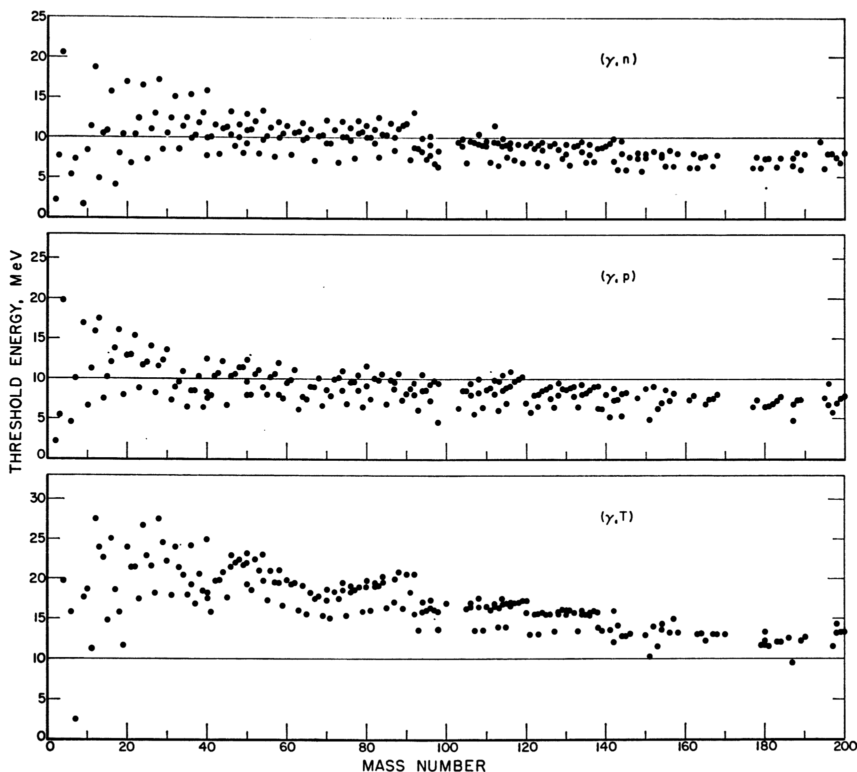

Figure 2. Threshold energies for photoneutron, photoproton, and phototriton reactions as a function of mass number (2)

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of microcuries per gram of element and 5 megarads as a function of the radioactivity half-life.

The specific radioactivities all fall within a band which decreases inversely with the half-lives. Generally, the light elements give the smallest specific activities and the heavy elements the largest. Fortunately, the longer one waits after processing, the smaller is the food handling as well as

Table I. Photoneutron, Photoproton, and Phototriton Threshold Energies Below 10 m.e.v. (*2*)

Stable Isotope	(γ,n)	(γ,p)	(γ,T)	*Stable Isotope*	(γ,n)	(γ,p)	(γ,T)
^{2}H	2.2	2.2		^{54}Fe		8.9	
^{3}He	7.7	5.5		^{57}Fe	7.6		
^{6}Li	5.4	4.6		^{59}Co		7.4	
^{7}Li	7.3		2.5	^{58}Ni		7.9	
^{9}Be	1.7			^{60}Ni		9.5	
^{10}B	8.4	6.6		^{61}Ni	7.8	9.8	
^{13}C	4.9			^{64}Ni	9.7		
^{14}N		7.5		^{63}Cu		6.1	
^{17}O	4.1			^{65}Cu	9.9	7.4	
^{18}O	8.1			^{64}Zn		7.7	
^{19}F		7.9		^{66}Zn		8.9	
^{21}Ne	6.8			^{67}Zn	7.1	8.9	
^{23}Na		8.8		^{70}Zn	9.2		
^{25}Mg	7.3			^{69}Ga		6.6	
^{27}Al		8.2		^{71}Ga	9.2	7.8	
^{29}Si	8.5			^{70}Ge		8.6	
^{31}P		7.3		^{72}Ge		9.9	
^{32}S		8.9		^{73}Ge	6.8		
^{33}S	8.6	9.6		^{76}Ge	9.6		
^{36}S	9.9			^{75}As		6.8	
^{35}Cl		6.4		^{74}Se		8.5	
^{37}Cl		8.4		^{76}Se		9.5	
^{36}Ar		8.5		^{77}Se	7.4	9.6	
^{40}Ar	9.9			^{82}Se	9.3		
^{39}K		6.4		^{79}Br		6.4	
^{40}K	7.8	7.6		^{81}Br		7.4	
^{41}K		7.8		^{78}Kr		8.5	
^{40}Ca		8.3		^{80}Kr		9.1	
^{43}Ca	7.9			^{83}Kr	7.5	9.8	
^{45}Sc		6.7		^{85}Rb		6.8	
^{47}Ti	8.9			^{87}Rb		8.7	
^{49}Ti	8.1			^{86}Sr		9.8	
^{50}V	9.3	7.9		^{87}Sr	8.4	9.5	
^{51}V		8.0		^{89}Y		7.3	
^{50}Cr		9.6		^{90}Zr		8.1	
^{53}Cr	7.9			^{91}Zr	7.2	8.7	
^{54}Cr	9.7			^{92}Zr	8.7	9.4	
^{55}Mn		8.0		^{94}Zr	8.2		

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch007

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Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

Table I. Continued

Stable Isotope	(γ,n)	(γ,p)	(γ,T)
^{96}Zr	7.8		
^{93}Nb	8.7	6.0	
^{92}Mo		7.9	
^{94}Mo	9.8	8.5	
^{95}Mo	7.3	8.6	
^{96}Mo	9.1	9.3	
^{97}Mo	6.7	9.7	
^{98}Mo	8.3	9.4	
^{96}Ru		7.2	
^{98}Ru	6.3	4.6	
^{104}Ru	8.9		
^{103}Rh	9.4	6.3	
^{104}Pd	9.8	8.5	
^{105}Pd	6.8	8.5	
^{106}Pd	9.6	9.4	
^{108}Pd	9.2	9.9	
^{110}Pd	9.0		
^{107}Ag	9.4	5.6	
^{109}Ag	9.1	6.4	
^{106}Cd		7.9	
^{108}Cd		8.2	
^{110}Cd	9.6	8.6	
^{111}Cd	6.9	8.8	
^{112}Cd	9.5	9.8	
^{113}Cd	6.5	9.7	
^{114}Cd	9.0		
^{116}Cd	8.7		
^{113}In	9.3	6.0	
^{115}In	9.1	6.9	
^{112}Sn		8.1	
^{114}Sn	9.8	8.6	
^{115}Sn	7.6	8.8	
^{116}Sn	9.4	9.1	
^{117}Sn	7.2	9.7	
^{118}Sn	9.2	9.9	
^{119}Sn	6.8		
^{120}Sn	9.0		
^{122}Sn	8.7		
^{124}Sn	8.4		
^{121}Sb	9.3	5.8	
^{123}Sb	9.0	6.6	
^{120}Te		6.9	
^{122}Te		7.9	
^{123}Te	6.9	8.1	
^{124}Te	9.4	8.5	
^{125}Te	6.5	8.7	
^{126}Te	9.0	9.0	
^{128}Te	8.6	9.4	
^{130}Te	7.9		
^{127}J	9.3	6.4	
^{126}Xe		7.5	
^{128}Xe		8.0	
^{129}Xe	7.4	8.7	
^{130}Xe	9.2	8.6	
^{131}Xe	6.6	8.8	
^{132}Xe	8.9	9.1	
^{134}Xe	8.3	9.3	
^{136}Xe	7.9		
^{133}Cs	9.1	6.4	
^{132}Ba		7.7	
^{134}Ba	9.5	8.0	
^{135}Ba	6.9	8.2	
^{136}Ba	9.2	8.6	
^{137}Ba	7.0	9.0	
^{138}Ba	8.7	9.1	
^{138}La		6.2	
^{139}La	8.7	6.2	
^{140}Ce	9.0	8.0	
^{142}Ce	7.1	8.7	
^{141}Pr	9.3	5.2	
^{142}Nd	9.8	7.3	
^{143}Nd	6.0	7.4	
^{144}Nd	8.0	8.1	
^{145}Nd	6.0	8.2	
^{146}Nd	7.5		
^{148}Nd	7.4		
^{150}Nd	7.4		
^{144}Sm	9.6	5.3	
^{148}Sm	8.1	7.6	
^{149}Sm	5.8		
^{150}Sm	8.0	8.7	
^{152}Sm	8.3	9.0	
^{154}Sm	7.6		
^{151}Eu		4.8	
^{153}Eu		6.2	
^{154}Gd	7.9	6.9	
^{155}Gd	6.4	8.6	
^{156}Gd	8.4	7.2	
^{157}Gd	6.4	8.1	
^{158}Gd	8.0		
^{161}Dy	6.2	7.3	
^{162}Dy	8.0	7.8	
^{163}Dy	6.2		
^{164}Dy	7.6		
^{165}Ho	7.7	6.8	
^{166}Er		7.4	

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Table I. Continued

Stable Isotope	(γ,n)	(γ,p)	(γ,T)
^{167}Er	6.4	7.5	
^{168}Er	7.8	8.0	
^{177}Hf	6.2	6.4	
^{178}Hf	7.5	7.3	
^{179}Hf	6.2		
^{180}Hf	7.3		
^{180}Ta		6.4	
^{181}Ta	7.4	6.6	
^{182}W		6.8	
^{183}W	6.3	7.2	
^{184}W	7.4	7.7	
^{187}Re	7.4	4.7	9.5
^{187}Os	6.4	6.7	
^{188}Os	8.1	7.3	
^{189}Os	6.0	7.3	
^{190}Os	7.9		
^{194}Pt	9.5		
^{195}Pt	6.1	7.6	
^{196}Pt	8.0	9.3	
^{198}Pt	7.6		
^{197}Au	8.0	5.8	
^{196}Hg		6.7	
^{198}Hg		6.8	
^{199}Hg	6.9	7.4	
^{200}Hg	8.1	7.8	
^{201}Hg	6.5	8.0	
^{202}Hg	7.9	8.6	
^{204}Hg	7.4		
^{203}Tl	8.8	6.1	
^{205}Tl	7.6	6.6	
^{204}Pb	8.5	6.6	
^{206}Pb	8.0	7.2	
^{207}Pb	6.7	7.5	
^{208}Pb	7.4	8.0	
^{209}Bi	7.4	3.7	9.4

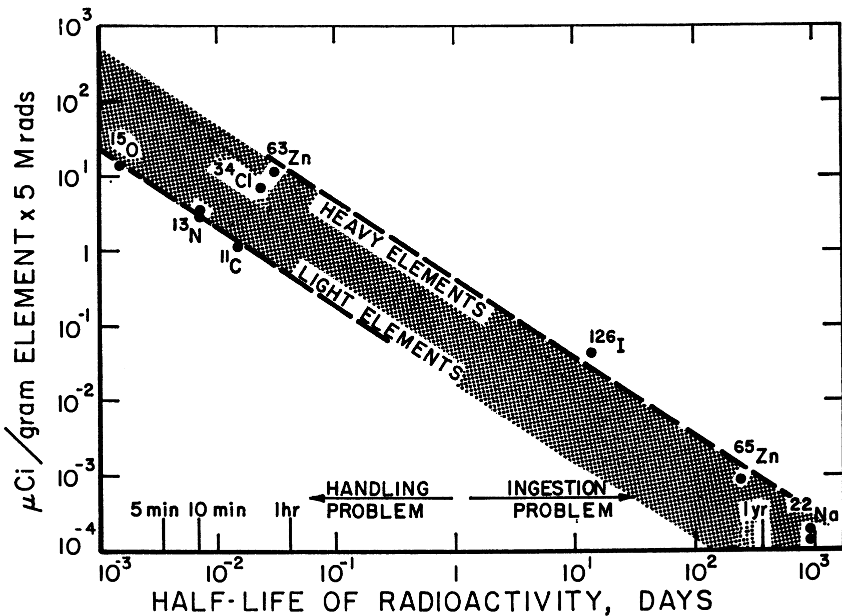

Figure 3. Relationship between specific activity and half-life of various radionuclides produced by a 5-megarad dose (9)

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Table II. Abundances of Elements in Beef (*9*)

Element	*Percent*	*Mg./100 grams*
H	9.3	9.3×10^3
B	6×10^{-6}	6×10^{-3}
C	18	1.8×10^4
N	1.5	1.5×10^3
O	70	7.0×10^4
Na	0.07	66
Mg	0.03	32
Al	1×10^{-6}	1×10^{-3}
Si	6×10^{-6}	6×10^{-3}
P	0.20	200
S	0.22	220
Cl	0.06	56
K	0.38	380
Ca	0.01	14
Cr	3×10^{-7}	3×10^{-4}
Mn	2×10^{-5}	2×10^{-2}
Fe	4.2×10^{-3}	4.2
Co	2×10^{-7}	2×10^{-4}
Ni	3×10^{-7}	3×10^{-4}
Cu	5×10^{-5}	5×10^{-2}
Zn	1.5×10^{-3}	1.5
Mo	1.5×10^{-6}	1.5×10^{-3}
Ag	1.4×10^{-6}	1.4×10^{-3}
Sn	2×10^{-7}	2×10^{-4}
I	3.5×10^{-6}	3.5×10^{-3}
Pb	1×10^{-6}	1×10^{-3}

the ingestion problem; fortunately, also, the high specific activities are associated with high sterilization doses and long storage times.

The general characteristics described above for the various elements can now be combined with the relative abundances of the elements in various foods in order to predict the radioactive concentrations in those foods. Table II shows the abundances of various elements found in beef (*9*). As can be seen and is generally known, the higher atomic-numbered elements are less abundant than those around carbon. Combining the results of Figure 3 and data from Table II yields decay curves for beef irradiated to 5 megarads, as plotted in Figure 4 (*9*). As shown in this figure, the total radioactivity resulting from photonuclear reactions can be analyzed into activities owing to ^{22}Na, ^{55}Fe, ^{65}Zn, etc. A more complete listing of the long-lived radionuclides resulting from nuclear reactions in various elements is given in Table III (*9*).

Two of these reactions are important and can be used as examples: the production of ^{22}Na by a (γ,n) reaction on ^{23}Na and of ^{24}Na by a neutron capture on ^{23}Na. The radioactivity emitted from ^{22}Na includes gamma radiation with energies of 1.25 and 0.51 m.e.v. and a half-life of 2.58 years; for ^{24}Na it includes gamma radiation with energies of 2.75 and 1.37 m.e.v.

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and a half-life of 14.97 hours. The x-ray threshold energy for the (γ,n) reaction producing ^{22}Na is 12.4 m.e.v. while the neutron threshold energy for the (n,γ) reaction producing ^{24}Na is thermal. Figure 5 illustrates the results of Equation 1 for ^{22}Na production in ham and beef as a function of the bombarding electron energy (*9, 15*), with a 5-megarad dose. Similarly, Figure 6 shows the data for ^{24}Na production (*9, 15*).

For completeness and comparison, the data in Table IV show the magnitudes of the activities induced by isomer activation in various elements in beef by 4-, 8-, 16-, and 24-m.e.v. x-rays (*15*). Activities are produced and measured at energies below 10 m.e.v. This production of isomers can also be initiated in principle by cobalt-60 γ-rays.

The specific examples of radioactivity production selected for this report are those that appear to be the most important on the basis of measurability and radiological significance. Continued research should be done on radioactivity production in foods to explore all the various possible

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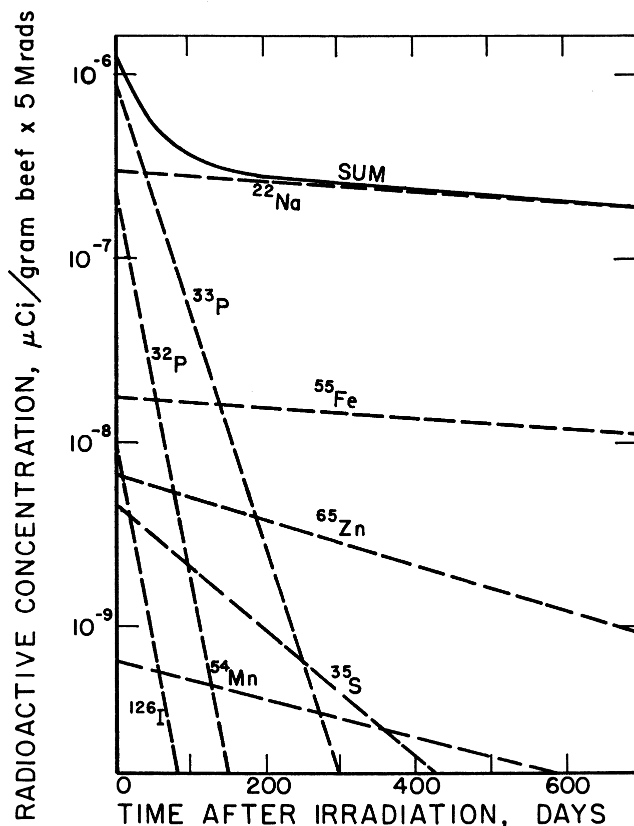

Figure 4. Decay curves for radioactivity induced in beef processed to a 5-megarad dose (9)

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

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Table III. Possible Long-Lived Radionuclides from Various Nuclear Reactions with Half-Lives in the Range of 10 Days to 10 Years (*9*)

Element	(γ,n)	(γ,p)	$(\gamma,pn)+$ (γ,d)	$(\gamma,2n)$	(n,γ)	(γ,T)	(γ,α)	(γ,γ')
H								
B						^{7}Be		
C								
N								
O								
Na	^{22}Na							
Mg			^{22}Na			^{22}Na		
Al								
Si								
P					^{32}P			
S	^{35}S	^{32}P	^{32}P		^{35}S			
		^{33}P						
Cl			^{35}S				^{33}P	
K			^{37}A					
Ca	^{45}Ca				^{45}Ca		^{42}A	
Mn	^{54}Mn							
Fe	^{55}Fe		^{54}Mn	^{55}Fe	^{55}Fe			
					^{59}Fe			
Co	^{58}Co			^{57}Co	^{60}Co			
Ni		^{57}Co	^{56}Co					
		^{60}Co	^{58}Co					
Cu	^{65}Zn			^{65}Zn	^{65}Zn			
Zn	^{86}Rb			^{83}Rb	^{86}Rb			
Rb	^{84}Rb							
Mo	^{93}Mo	^{91}Nb	^{92}Nb	^{93}Mo	^{93}Mo	^{95}Nb	^{88}Zr	
		^{95}Nb	^{95}Nb			^{92}Nb		
						^{91}Nb		
Ag				^{105}Ag	^{110m}Ag			
Sn	^{113}Sn	^{114m}In	^{114m}In	^{113}Sn	^{113}Sn	^{114m}In	^{115}Cd	^{117m}Sn
	^{121m}Sn				^{112}Sn			^{119m}Sn
	^{123}Sn				^{123}Sn			
I	^{126}I		^{125}Te	^{125}I				
			^{202}Tl			^{204}Tl	^{203}Hg	
			^{204}Tl					

parameters and processes. For example, if the maximum energy permissible for food processing is raised above 10 m.e.v., the question will be: how high shall it be raised? Energies as high as 30 m.e.v. are conceivably permissible. However, the present authors expect that above 30 m.e.v., processes such as ^{12}C$(\gamma,\alpha n)^{7}$Be, which have unusually high probabilities (*1*), must be explored in detail and may set an absolute upper limit to the permissible energies.

In addition, processes not considered previously deserve scrutiny, such as the emission from containers of recoil nuclei of tin-117m that are γ-ray

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

emitters in isomeric transitions. (If the container had an inner plastic liner, heavy recoil nuclei would be absorbed by the liner and therefore would not enter the contents.) Also, $^{14}N(n,T)^{12}C$ has a low neutron threshold and a high probability and could present difficulties under certain conditions.

Measurement Capabilities

The ability to measure an added radioactivity in food depends on the type of radiation (β-ray, positron, γ-ray), the energy of the radiation, the concentration of the radioactivity, the chemical characteristics of the radioactive element, and the chemical characteristics of the food. In other words, the measurement capability depends on the detector sensitivity for the specific radionuclides involved and on the background radioactivity in the chemically separated sample to be counted.

Detector Sensitivity. Detectors for nuclear radiation have been under rapid and continuous development during the last decade (*12*). The de-

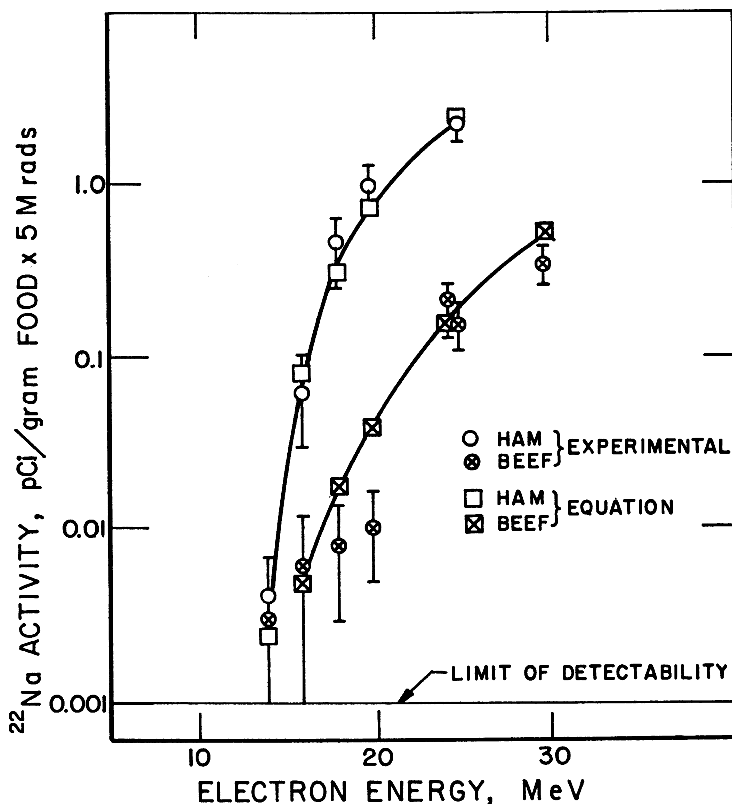

Figure 5. Production of ^{22}Na activity in ham and beef as a function of electron energy (15)

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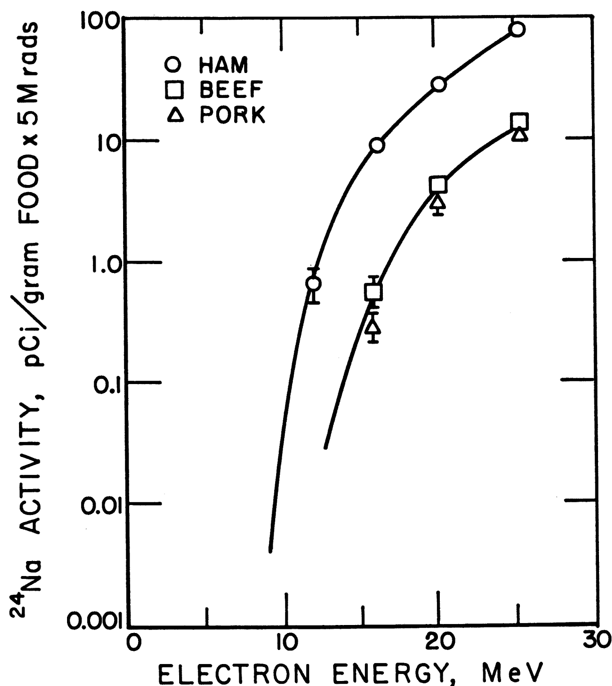

Figure 6. Production of ^{24}Na activity in ham, beef, and pork as a function of electron energy (15)

velopments have resulted in the ability under certain specialized conditions to detect almost every β-ray, positron, or γ-ray emitted in a nuclear disintegration. Two particular extreme situations can illustrate the sensitivity of detectors.

GAMMA RAYS (NUCLEAR-DECAY γ-RAYS, 0.5-M.E.V. PHOTONS FROM ANNIHILATION OF POSITRONS OR X-RAYS). The development of large sodium iodide crystal γ-ray spectrometers (*13*) has made possible high detection efficiencies (close to 100% for some γ-ray energies). Also, whole-body counters utilizing large cylindrical liquid scintillators provide a detection efficiency of 15% for the γ-rays emitted from potassium-40 in the human body (*23*).

It is clear from the example of the whole-body counter that even for a large sample, the detection efficiency of γ-rays is quite large and that the ultimate sensitivity strongly depends on the background counting rates above which the radioactivity of interest is being observed. The ultimate sensitivity is also determined by practical counting rates of a few counts per minute. Therefore, assuming no background, the ultimate sensitivity is of the order of 2 disintegrations per minute, or 10^{-12} curie—i.e., 1 pico-

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Table IV. Experimentally Determined Radioactivity-Produced from Isomer Activation (*15*) (Activity in pCi/gram beef × 5 mrads)

		X-Ray Energy, m.e.v.			
Isomer	*Half-life*	*4*	*8*	*16*	*24*
^{87m}Sr	2.8h	3×10^{-5}	5×10^{-4}	0.8	1
^{93m}Nb	12y	—	—	—	—
^{115m}In	4.5h	5×10^{-5}	9×10^{-3}	0.08	0.14
^{123m}Te	104d	—	—	8.8×10^{-10}	—
^{125m}Te	58d	—	—	—	2×10^{-8}
^{135m}Ba	28.7h	2×10^{-6}	2×10^{-4}	7×10^{-3}	0.02
^{204m}Pb	67m	2×10^{-7}	—	0.03	0.03
^{160m}Hf	5.5h	5×10^{-7}	6×10^{-8}	—	—
^{195m}Pt	3.5d	—	—	—	—
^{199m}Hg	42m	1.8×10^{-9}	3×10^{-2}	—	—

curie or 1 pCi. For γ-ray detectors, the practical sample sizes are of the order of 100 grams. Therefore, the sensitivity is:

$$\frac{1 \text{ pCi}}{100 \text{ grams}} = 10^{-2} \frac{\text{pCi}}{\text{gram}}$$

Since the detection efficiencies can be of the order of 15% or better and since there are approximately 4×10^{22} atoms per gram, the above sensitivity for a radioactivity with a half-life of one month means a few nuclei of the radioactive species in 10^{17} other nuclei. As Meyer points out (*15*), "this is an incomparably greater sensitivity to any potentially carcinogenic additives than other means such as chemical analysis which yields impurities in parts per 10^6 or spectroscopic means with a maximum sensitivity of approximately one part in 10^9" (*13*).

TRITIUM, CARBON-14, AND STRONTIUM-90. The other extreme of detectability might be thought to be low energy betas from hydrogen-3. Hydrogen-3 (tritium) emits an 18.6 k.e.v. β-ray with a half-life of 12.26 years (no γ-rays). The sample sizes here must be small to permit detection of the β-rays by the apparatus. Nevertheless, the detection sensitivities for these radioactivities are of the order of 10^{-3} pCi per gram.

These detection sensitivities are small compared with the magnitude of radioactivity generated and readily observed in foods, as seen in Figures 5 and 6.

Background. The background radioactivity in food arises from natural causes and radioactive fallout. The magnitude of this activity is indicated in Table V (*5, 15, 21*).

The ability to observe added radioactivity in a food sample will depend on the inherent background counting rate of the sample and the ability to reduce the background activity relative to the added activity under ob-

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Table V. Background Radioactivity in Food (pCi/gram)

Natural	^{3}H,	3.6	^{40}K,	3.0
	^{14}C,	1.3	^{226}Ra,	8×10^{-4}
Fallout	^{90}Sr,	1.7×10^{-2}	^{137}Cs,	7×10^{-2}

servation. Substantial improvement in the capability to measure an added radioactivity can be accomplished by (*15*):

METHOD 1. Post irradiation ashing of the sample and counting of the residue and chemical separation which will achieve considerable increase in specificity for some elements.

METHOD 2. Adding known amounts of elements to the food prior to irradiation.

METHOD 3. Irradiating aqueous solutions of the elements known to be trace constituents of food.

METHOD 4. Improving the specificity of the detection equipment to the type and energy of the emitted radiations.

Meyer states that the minimum detectability of straight counting of a sample in its original physical state is a few picocuries per gram whereas the minimum detectability of the above methods is: Method 1, 0.003 pCi per gram, and Methods 2 and 3, 10^{-6} pCi per gram.

Future Improvements in Detection and Measurement of Radioactivity. Future techniques will result in a greater degree of specificity in the observation of radioactivity in foods. By using much-improved γ-ray spectrometers employing lithium-drifted germanium detectors (*6*) and using multidimensional pulse height analyzers in on-line computers (*18*), greater sensitivities and ease of measurement will be afforded in the future.

In summary, the techniques and measurement capabilities to detect extremely small quantities of radioactivity are highly developed, but improvements are still being made. It is expected that measurable radioactivity could be found in food irradiated at energies below 10 m.e.v. if the ultimate in detection techniques were employed and if large samples and measurements immediately after the irradiation were used.

Radiological Significance of Radioactivity Production

The significance of radioactivity in water, food, and air has been under particular scrutiny since the advent of nuclear bomb tests and their accompanying radioactive fallout. This scrutiny has resulted in much documented data gathered over the past few decades on the health characteristics of man under various environmental conditions, such as: the Denver populace who are subjected to more intense cosmic ray irradiations than the New York City populace; the radium dial workers of the 1920's who have provided data on life spans, general health, and causes of death; population's drinking water with varying radium contents; persons living

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in brick houses *vs.* those living in frame houses; the Hiroshima bomb victims; radiation *vs.* nonradiation workers.

One result of studies on these persons with varying radiation histories has been the development of criteria on the maximum permissible concentrations of radioactivity in air and in water ingested by the population. The criteria factors that determine the hazards of the various radionuclides include:

1. The particular radionuclides involved.
2. Initial body retention.
3. Fraction going from blood to critical body tissue.
4. Radiosensitivity of tissue.
5. Size of critical organ.
6. Essentiality of the critical organ to the proper function of the body.
7. Biological half-life.
8. Radioactive half-life.
9. Energy of the radiation produced by the radionuclide.

These factors have been used to develop the tables of maximum permissible concentrations (*16*) in air and in water for various conditions (40-hour week or 168-hour week) and for various organs (skin, bone, kidney, liver, brain, GI tract, and total body), but all assuming an occupational exposure and an exposure period of 50 years. MPC values have been developed for 234 radionuclides found in nature, in radioactive fallout, and in neutron activation studies.

The MPC values for water and for a 168-hour week can be applied to foods if appropriate care is taken—for example, the MPC values assume an average daily intake of 2200 grams of water for a standard man. If 2200 grams of radioactive food are consumed every day for 50 years, the referenced MPC values for water are applicable. This situation is obviously not the case, and correction factors that account for the intake of various foods are needed. Intake estimates are obtainable from data such as shown in Table VI (*15*). The correction factor is roughly the ratio of 2200 grams to the grams of daily intake of the irradiated or radioactive food.

Table VI. Average Intake of Food per Day in United States (*15*)

Component	*% Total Weight*
Water	33.1
Meat, fish, and poultry	14.5
Milk products	14.0
Root vegetables	10.6
Leafy, green, and other vegetables	8.9
Fruit	8.2
Cereal and grain	7.3
Eggs	3.5
Beverages (tea and coffee)	0.03

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Another important consideration is the recommendation of a reduction factor in MPC values for nonradiation workers compared with the values applicable to radiation workers. A factor of one-tenth is recommended for nonradiation workers (*16*). An additional factor is usually suggested to accommodate the most sensitive segments of the population. Children require special attention.

All of the factors alluded to above should be considered to develop appropriate MPC values for irradiated foods to be consumed by the general public. These values are presently not available.

Additionally, a number of the radionuclides that can be produced by photonuclear reactions do not appear in the available MPC tables (*16*). E. G. Fuller, National Bureau of Standards, has prepared a complete table of radionuclides that could be produced in radiation processing. K. Z.

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Table VII. Partial Listing of Radionuclides Generated in Radiation Processing for which MPC values have not yet been Established (Half-Life Values in Range of 1/2 Hour to 10^5 Years)

Radionuclide	*Half-life*	*Radiation*	*Reaction*	*Parent*	*% Abundance of Parent*
^{13}N	10.0m	$\beta+$	γ,n	^{14}N	99.6
^{15}O	2.1m	$\beta+$	γ,n	^{16}O	99.76
^{33}P	25d	$\beta-$	γ,p	^{34}S	4.22
^{34}Cl	32m	$\beta+,\gamma$	γ,n	^{35}Cl	75.5
^{39}Cl	56m	$\beta-,\gamma$	γ,p	^{40}A	99.6
^{39}A	265y	$\beta-$	γ,n	^{40}A	99.6
^{43}K	22h	$\beta-,\gamma$	γ,p	^{44}Ca	2.06
^{44}Sc	2.4d	γ	γ,n	^{45}Sc	100.0
	3.9h	$\beta+,\gamma$	γ,n		
^{49}Sc	58m	$\beta-$	γ,p	^{50}Ti	5.34
^{49}V	330d	K	γ,p	^{50}Cr	4.3
			γ,n	^{50}V	0.24
^{49}Cr	42m	$\beta+,\gamma$	γ,n	^{50}Cr	4.3
^{56}Co	77.3d	K,$\beta+,\gamma$	γ,d	^{58}Ni	67.9
^{57}Ni	37h	$\beta+,\gamma$	γ,n	^{58}Ni	67.9
^{63}Zn	38m	$\beta+,\gamma$	γ,n	^{64}Zn	48.9
^{68}Ga	68m	$\beta+,\gamma$	γ,n	^{69}Ga	60.4
^{69}Ge	40h	K,$\beta+,\gamma$	γ,n	^{70}Ge	20.5
^{75}Ge	82m	$\beta-,\gamma$	n,γ	^{74}Ge	36.5
			γ,n	^{76}Ge	7.8
^{73}Se	44m	$\beta+,\gamma$	γ,n	^{74}Se	0.9
	7.1h	$\beta+,\gamma$			
^{74}Se	120d	K,γ	γ,n	^{75}Se	9.0
^{81}Se	57m	γ	n,γ	^{80}Se	49.8
^{80}Br	4.5h	γ	γ,n	^{81}Br	49.5
^{77}Kr	1.2h	$\beta+,\gamma$	γ,n	^{78}Kr	0.3
^{79}Kr	34.5h	K,$\beta+,\gamma$	γ,n	^{80}Kr	2.3
^{83}Kr	1.9h	γ	γ,n	^{84}Kr	56.9
^{84}Rb	33d	$\beta+,\beta-,\gamma$	γ,n	^{85}Rb	72.1

Table VII. Continued

Radionuclide	*Half-life*	*Radiation*	*Reaction*	*Parent*	*% Abundance of Parent*
^{83}Sr	34h	K,β+,γ	γ,*n*	^{84}Sr	0.6
^{87}Sr	2.8h	γ	γ,*n*	^{88}Sr	82.6
^{88}Y	105d	K,β+,γ	γ,*n*	^{89}Y	100.0
^{89}Zr	79h	K,β+	γ,*n*	^{90}Zr	51.5
^{91}Nb	62d	K,γ	γ,*p*	^{92}Mo	15.8
^{92}Nb	3.2h	γ	γ,*n*	^{93}Nb	100.0
	10.2d	K,β+,γ	γ,*d*	^{94}Mo	9.0
^{90}Mo	6h	β+,γ,K	γ,2*n*	^{92}Mo	15.8
^{93}Mo	6.9h	γ	*n*,γ	^{92}Mo	15.8
			γ,*n*	^{94}Mo	9.0
			γ,2*n*	^{95}Mo	15.7
^{94}Ru	1h	K	γ,2*n*	^{96}Ru	5.57
^{95}Ru	100m	K,β+,γ	γ,*n*	^{96}Ru	5.57
^{101}Rh	5y	γ	γ,2*n*	^{103}Rh	100.0
	4.52d	K,γ			
^{102}Rh	210d	K,β−,β+,γ	γ,*n*	^{103}Rh	100.0
^{100}Pd	4.1d	K,γ	γ,2*n*	^{102}Pd	1.0
^{101}Pd	8.5h	K,β+,γ	γ,*n*	^{102}Pd	1.0
^{111}Pd	5.5h	β−,γ	*n*,γ	^{110}Pd	11.8
^{106}Ag	8.3d	K,γ	γ,*n*	^{107}Ag	51.4
^{104}Cd	59m	K,γ	γ,2*n*	^{106}Cd	1.22
^{105}Cd	55m	K,β+,γ,e^-	γ,*n*	^{106}Cd	1.22
^{107}Cd	6.7h	K,β+,γ	*n*,γ	^{106}Cd	1.22
			γ,*n*	^{108}Cd	0.88
^{111m}Cd	49m	e^-	*n*,γ	^{110}Cd	12.4
			γ,γ	^{111}Cd	12.8
			γ,*n*	^{112}Cd	24.0
			γ,2*n*	^{113}Cd	12.3
^{113m}Cd	5.1y	β−	*n*,γ	^{112}Cd	24.0
			γ,γ	^{113}Cd	12.3
			γ,*n*	^{114}Cd	28.8
^{117}Cd	3.0h	β−,γ	*n*,γ	^{116}Cd	7.6
	50m	β−,γ			
^{111}In	2.8d	K,e^-,γ	γ,2*n*	^{113}In	4.33
^{110}Sn	4.0h	K,γ	γ,2*n*	^{112}Sn	1.02
^{111}Sn	35m	K,β+	γ,*n*	^{112}Sn	1.02
^{117m}Sn	14d	γ,e^-	*n*,γ	^{116}Sn	14.3
			γ,γ	^{117}Sn	7.6
			γ,*n*	^{118}Sn	24.1
			γ,2*n*	^{119}Sn	8.5
^{119m}Sn	245d	γ,e^-	*n*,γ	^{118}Sn	24.1
			γ,γ	^{119}Sn	8.5
			γ,*n*	^{120}Sn	32.5
^{121}Sn	5y	β−,γ	*n*,γ	^{120}Sn	32.5
	27h	β−	γ,*n*	^{122}Sn	4.8
^{123}Sn	130d	β−,γ	*n*,γ	^{122}Sn	4.8
	40m	β−,γ	γ,*n*	^{124}Sn	6.1

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Morgan and co-workers at the Oak Ridge National Laboratory have indicated particular radionuclides for which MPC values could and will be developed. Since the radioactivity produced by photonuclear reactions is in principle no different in carcinogenicity or general health effects than radioactivity resulting from other causes, the same criteria used to develop the available MPC values are applicable to the radioactivity generated in radiation processing. A formal request has been made by the National Bureau of Standards of the National Council on Radiation Protection and Measurements to develop the necessary MPC values. Examples of the radionuclides for which MPC values are being developed are listed in Table VII. The preliminary work on these values has been completed.

To illustrate the application of MPC values and the relative amounts of radioactivity that would be ingested by consuming a radiation-processed food *vs.* water containing the maximum permissible concentration, consider the following example. From the data provided in Figure 5, the induced ^{22}Na resulting from processing ham with a 5-megarad dose using 24-m.e.v. electrons is approximately 1.4 pCi per gram. The MPC of ^{22}Na in water should be about 40 pCi per cc. for nonradiation workers. Thus, if the complete daily diet were the irradiated ham, the amount of radioactivity ingested would be 1/28 of (1.4/40) that permitted to be ingested in an equivalent mass of water. Since irradiated ham would, in fact, be a small part of the normal diet, the MPC of ^{22}Na in ham could reasonably be higher than in water. Therefore, the induced ^{22}Na would result in a concentration much less than the reasonable MPC. Also, Meyer has shown (*15*) that the added radioactivity in most foods under these conditions is less than 5% of the natural activity already present before irradiation.

Reducing Radioactivity Production in Food Processing

If electron and/or x-ray energies higher than 10 m.e.v. are permitted in the future, procedures to minimize the small magnitudes of radioactivity generated will be useful. The following are points to be considered.

The thickness of the food container used for electron beam processing should be as thin and of as low an atomic number as possible. A thin aluminum flexible wrap is to be preferred to a thick steel can so as to minimize the production of radioactivity by x-rays resulting from electron interactions with the container and to reduce the absorption of the electron energy.

The sterilizing doses supplied by electrons are to be preferred to the same doses supplied by x-rays because of the greater probability for producing radioactivities by x-rays than by electrons.

Using two electron or x-ray beams of reduced energy (1/2 energy for the electron) is to be preferred over one beam of the maximum energy because the probabilities for producing radioactivities increase with energy.

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Attention should be given to a reduction in the neutron moderators located close to the package being processed because of the high probability for producing radioactivities by slow neutron absorption.

Allowance should be provided for as long a time period as possible after processing before the sterilized food is ingested in order to permit the induced radioactivities to decay.

Foods should be processed with the smallest dose magnitude required to obtain the desired effect of sterilization or pasteurization because of the proportionate increase in radioactivity production at the high dose magnitudes.

Acknowledgment

The authors acknowledge the valuable comments and suggestions of L. A. Currie, W. B. Mann, and H. O. Wyckoff regarding the production and measurement of radioactivity.

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RECEIVED November 9, 1965. Work partially supported by the Division of Isotopes Development, U. S. Atomic Energy Commission.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch007

8

Elements of Gamma Irradiator Design

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch008

Irradiation devices for destroying bacteria in packaged goods consist of a radioactive source, a conveyor, and shielding. For food irradiation the important design criteria include a high uniformity of treatment, relatively short irradiation times, and close temperature control. Three steps in making a ^{60}Co source are encapsulation of the cobalt, irradiation in a nuclear reactor, and secondary encapsulation along with appropriate testing and radiation assay. Advantages of high specific activity ^{60}Co—the number of encapsulations are reduced and the volume of the irradiator to be shielded is minimized—must be balanced against the cost of obtaining higher specific activities. For food pasteurization irradiators, design techniques are available today. For food sterilization applications considerably more design and source technology development is needed.

Gamma irradiators are simple, rugged, and reliable devices for destroying bacteria in packaged goods. About a dozen commercial units have now been built around the world, mainly for sterilizing medical supplies (*1*, *3*). They are all characterized by high on-stream times, low maintenance, and low labor costs.

An irradiator consists simply of a radioactive source, a conveyor, and shielding. In general, for package irradiators, the customer will specify the capacity (pounds per hour), the desired dose (rads), the desired uniformity of treatment (maximum-minimum ratio), the package dimensions, the package density, and the maximum residence time permitted in the unit. It is up to the irradiator designer to fix the conveyor speed, the source dimensions, the source specific activity, and the source-to-target configuration to optimize the efficiency and minimize the cost of the unit.

Two radioisotopes have the penetrating power and other characteristics that are useful for irradiation of food and medical supplies: ^{60}Co and ^{137}Cs. Of these ^{60}Co is in the most advanced technological state.

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

Design Methods

For food irradiation one of the most important design criteria is uniformity of treatment. In food sterilization, dose uniformities expressed as a ratio of maximum dose at any point in the package to the minimum dose at any point in the package must bear ratios of 1.25 or lower whereas for food pasteurization, ratios of 1.5 to 2.0 can be tolerated. The reason for these ratios is that if the dose is too low, not all of the bacteria will be destroyed whereas if the dose is too high, some secondary deleterious effects will occur, such as taste change. If one is to design for a maximum-minimum ratio of 1.25—i.e., variation of $\pm 12.5\%$—it must be possible to predict the dose at any point in the package with a precision much greater than the variation permitted. One would like to be able to predict the dose at any point in a finite target with an accuracy of $\pm 5\%$.

After several years of study at Brookhaven National Laboratory analytical methods have been developed which we believe permit one to predict dose distribution in finite targets from finite sources with the required degree of accuracy. These methods and computer codes have been published (*4*).

These design methods are not yet complete for they involve only simple geometries and simplified assumptions as to target homogeneity, but they go a long way in permitting one to design irradiators for various food applications with confidence.

Source Manufacture

At present ^{60}Co sources are made in England, Canada, and the United States. The primary source manufactured in the United States is the BNL standard source (*5*), which is in the form of a strip of cobalt metal about ¾ inch wide, 1 foot long, and 60 mils thick, doubly encapsulated in thin stainless steel jackets. The total amount of self-absorption of γ-rays in this source is less than 10%—i.e., over 90% of the γ-rays are available for absorption in target material.

There are three steps in manufacturing a ^{60}Co source: (1) preparing the cobalt metal strip with its primary encapsulation in an irradiation container, (2) irradiation in a nuclear reactor, and (3) providing of a secondary encapsulation along with appropriate leak testing and radiation assay.

Figures 1 and 2 illustrate the cobalt metal strips and their irradiation can. This manufacturing method was developed at Brookhaven. We have recently developed specifications for an improved metallurgically bonded stainless steel cobalt metal strip in a simplified container. These are now being manufactured commercially.

The nuclear reactors at Savannah River are being used for ^{60}Co irradiation. These production reactors are extremely well suited for this purpose since they have high fluxes and even flux distribution. Their capacity far

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch008

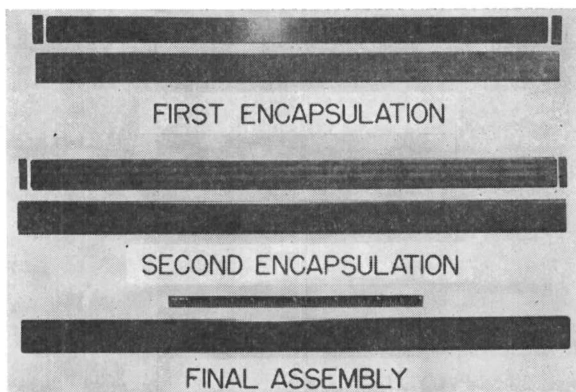

Figure 1. Standard source

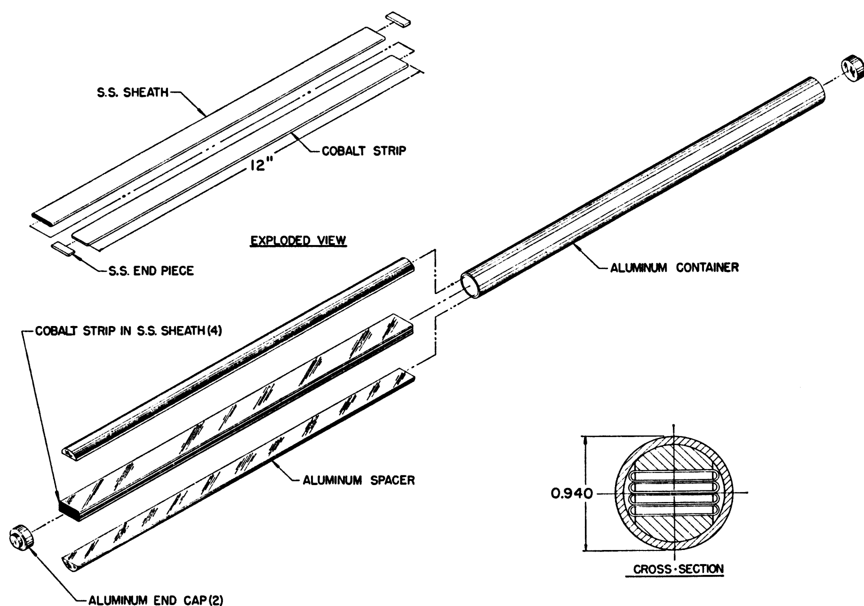

Figure 2. Exploded view of standard source

exceeds that needed for any contemplated commercial applications over the next decade.

In addition to Federal Government (U. S. Atomic Energy Commission, U. S. Army, Bureau of Mines, Bureau of Standards, etc.) requirements, several companies, including the American Novawood Corp., American Nuclear Corp., Gamma Process Co., General Electric Co., Lockheed Aircraft Corp., Nuclear Materials and Equipment Corp., Picker X-Ray Corp., and U. S. Nuclear Corp., and the Japanese and Canadian governments have

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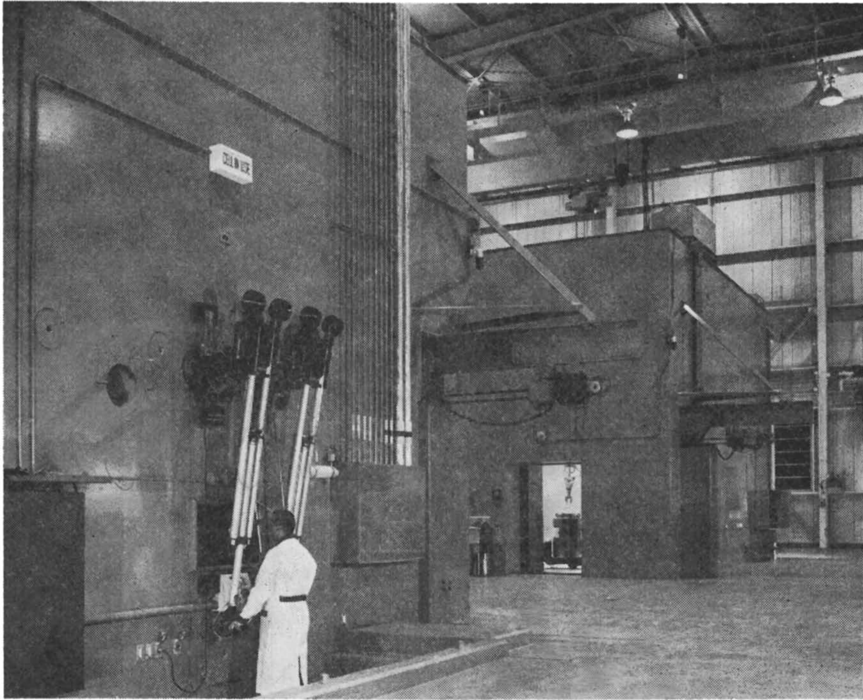

Figure 3. Facilities for second encapsulation

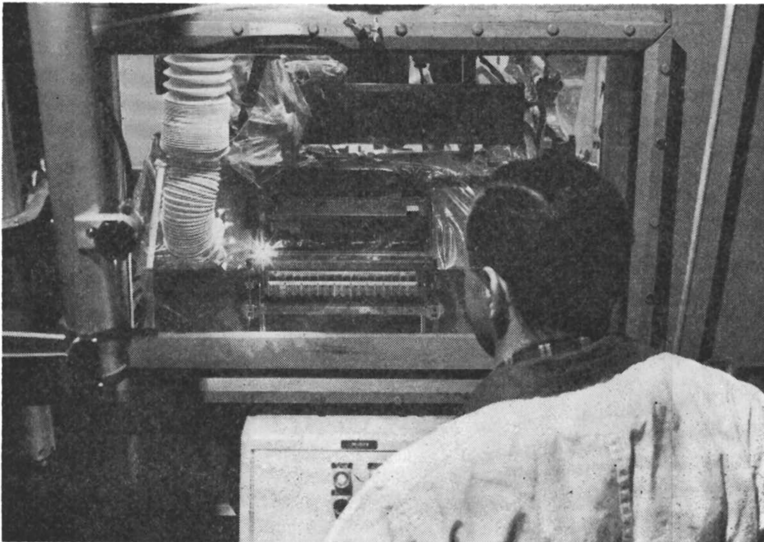

Figure 4. Facilities for second encapsulation

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

purchased or placed orders for 100,000-curie and greater lots of ^{60}Co from the AEC's Oak Ridge National Laboratory. Generally, these companies will act as brokers and service agencies for encapsulated and completely fabricated ^{60}Co sources.

The second encapsulation must be put on remotely and requires facilities such as shown in Figures 3 and 4. Methods for providing this remote encapsulation, including remote welding, remote leak testing, and remote radiation assay, were developed at Brookhaven. These techniques were made available to industry, and several of the above companies can now provide these services.

These doubly encapsulated strips are the building blocks for assembling plaques or other geometrical forms that are the gamma energy–emitting sources of food irradiators. Figure 5 illustrates one such plaque.

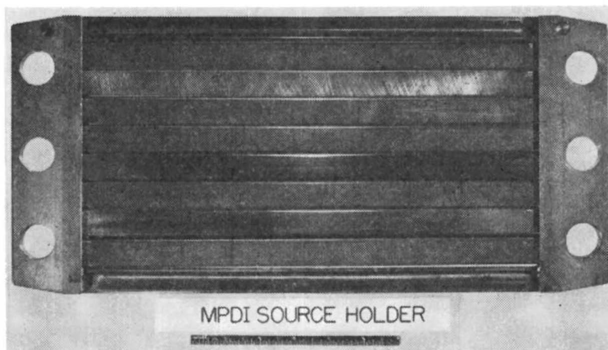

Figure 5. Doubly encapsulated strips

Specific Activity

Although the methods and materials described are adequate to construct commercial irradiators today, economic forces will ultimately require the use of higher specific activities than are used today. An example will perhaps illustrate this point. Suppose one desires to build an irradiator to pasteurize strawberries. Peak loads and therefore design loads would be about 10,000 pounds per hour. About 10 kw. of radiation power at reasonable over-all efficiencies, or about 670,000 curies of ^{60}Co, would be required. If the radiation source were made up at 2 curies per gram, one would require 4000 BNL strips in 1000 irradiation cans at a cost of ~\$100,000; at 20 curies per gram, 400 strips in 100 irradiation cans at a cost of ~\$10,400; and at 200 curies per gram, 40 strips in 10 irradiation cans at a cost of ~\$1600.

The cost of irradiation—i.e., the cost of the curies—depends greatly on which reactor the cobalt is irradiated in. If irradiated in a reactor at high flux, the high specific activity material would be irradiated in 10 cans for

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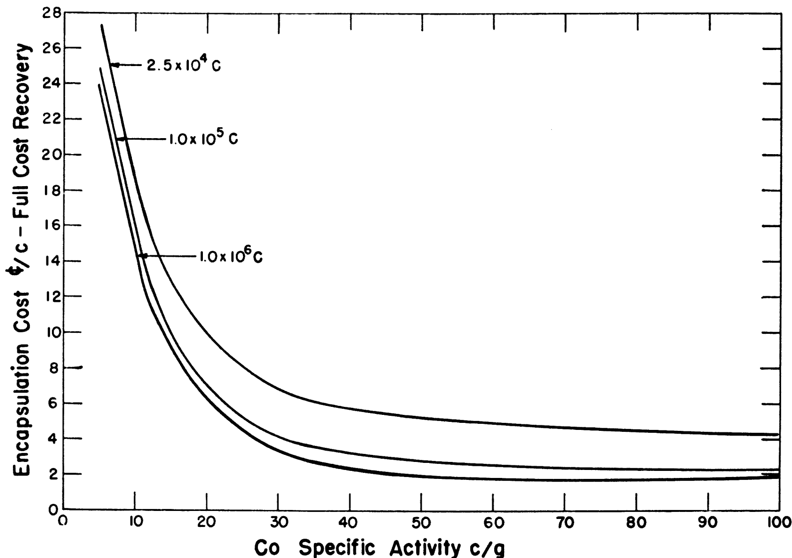

Figure 6. Effect of specific activity on cost

about one year. At intermediate fluxes the intermediate specific activity could be made in 100 cans in one year, and at low fluxes the low specific activity could be made in 1000 cans in one year. If done in this way, the cost of the curies is likely to be about the same. If done at Savannah River, it would actually be more difficult to handle 1000 cans for a short irradiation period than 10 cans for a long irradiation period.

The effect of specific activity on the cost of the remote second encapsulation is shown in Figure 6. Costs depend somewhat on batch size but are reduced dramatically by an increase in specific activity. The reasons for this are obvious from the example we chose. At 200 curies per gram we are remotely encapsulating only 40 strips; at 20 curies per gram, 400; and at 2 curies per gram, 4000. The second encapsulation costs for our 670,000-curie example would be approximately $13,500, $27,000, and $142,000 for the high to low specific activities, respectively.

Another dramatic effect is in the retention time in the irradiator cell and thus in irradiator cell costs. The high specific activity would be fabricated into a plaque only 2.4 feet long, the intermediate specific activity would require a plaque 24 feet long whereas the low specific activity would require a plaque 240 feet long. The strawberries would reside in the irradiator for one hour for the long plaque, 6 minutes for the intermediate, and 36 seconds for the short plaque. The cost for irradiator shielding would vary from about $20,000 for the high specific activity source, to about $30,000 for the intermediate activity, and to about $240,000 for the low specific activity.

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

High specific activity poses some problems of its own, however, including heat generation and dissipation in the source and target, energy storage in construction materials, and some radiation damage problems. These problems are under study and should be surmounted within the next few years.

Source-to-Target Configurations

A wide variety of source-to-target configurations is possible, and it depends upon the ingenuity of the designer to balance mechanical complexity with efficiency and with other parameters specified by the customer.

Figure 7 illustrates the Johnson plant at Slough, England, and Figure 8 shows the route of the monorail carriers through the Johnson plant (*2*). As can be seen, each package makes eight horizontal passes around the source and three vertical passes. By this sort of source-to-target configuration an efficiency of about 20% is obtained for a package density of about 0.25 gram per cc. Within the same cell and for the same attentuation distance a considerably greater efficiency could be obtained for a denser target up to a maximum efficiency of about 35%. However, to obtain the same uniformity of treatment, the package thickness of the denser product

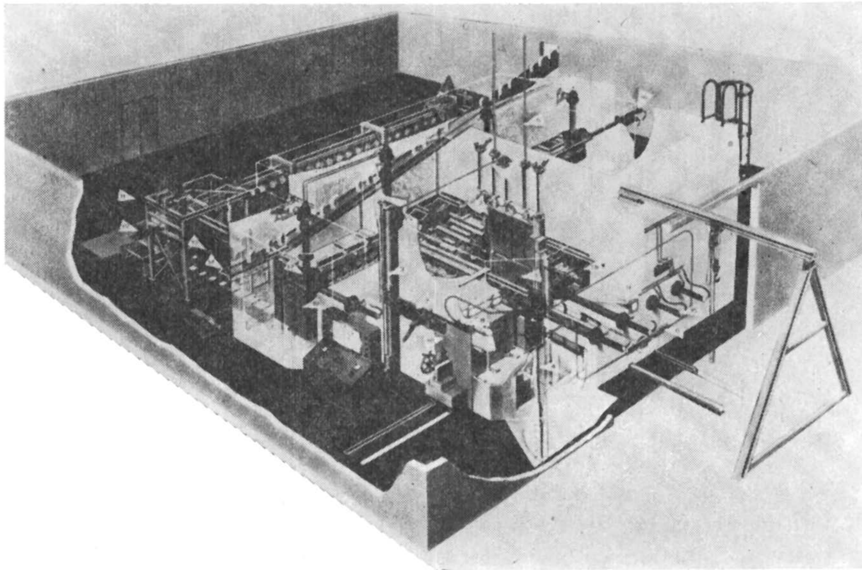

Figure 7. Johnson plant at Slough, England

Radioactive source, source storage shield plug, source hoist, source pit, source position indicator rods, loading port interlock, interlocks (doors to source hoist and to loading port), viewing window, plug door, package load/unload ram, package loading conveyor, tilting mechanism for load/unload of packages, unloading conveyor, longitudinal ram, cross-transfer ram, turntable, cross-transfer carrier, and package container

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch008

would have to be less, requiring more horizontal passes and thus more mechanical complexity.

These multipass systems generally require appreciable retention times within the cell (many hours). If retention time must be minimized, it may be necessary to go to a less efficient source to target geometry.

Much higher efficiencies can be obtained if the product can be handled in bulk rather than in packages. Thus, for a bulk grain irradiator or for liquid irradiations, efficiencies in the 40–70% region can be obtained.

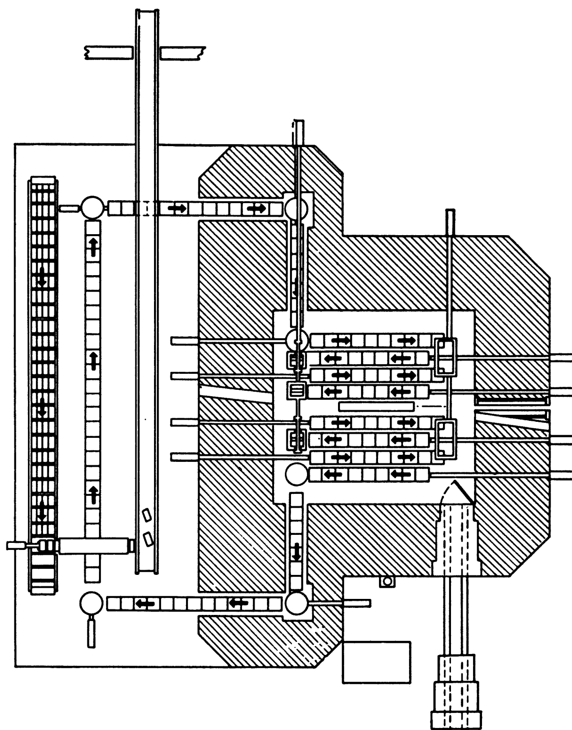

Figure 8. Route of monorail carriers through plant

In general, facilities, sources, and design techniques for food pasteurization are available commercially today or will be available in the near future to satisfy initial needs.

For food sterilization applications, considerably more design and source technology development is needed, particularly in predicting effects in heterogeneous targets.

Irradiator technology seems to be keeping pace with food irradiation research.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch008

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RECEIVED November 9, 1965.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch008

9

Establishing a Food Irradiation Facility, and Related Economic Aspects

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch009

The approach to selecting a radiation-processing facility parallels that for conventional or nonradiation plants. The determination and analyses of facility requirements for radiation-processed foods require knowledge of the conventional processing counterpart. In most cases, the irradiation must be made an integral part of the process and not merely an "extra step" plugged into an existing facility or process. The combination and relative importance of present or proposed product-handling techniques, together with the normal technological response patterns of foods involved, will dictate site location, facility size, throughput rates, and ultimate economics. The relationship of these production requirements is explored, with reference to facilities for pasteurization and sterilization of food products.

One of the drawbacks to greater use of ionizing radiation as a processing tool lies in the fact that too few individuals are familiar with the utilization of the fast-growing and versatile technology of the atomic age. There is a tendency to rely on improved conventional methods of production or processing, even when radiation can offer a distinct advantage. In defense of this type of reaction, it is not entirely unwise to choose a conventional, better-known process over a less familar one nor to produce or process a product better assured of public acceptance over one where consumer acceptability—perhaps even at lower prices—is unknown.

Nevertheless, the field of radiation processing is growing, and an increasing number of products or processing methods are achieving commercial status (*3*). With increasing awareness and confidence by both the processor and the consumer in the use and safety of radiation sources and facilities, as well as of the end product, the field of process radiation is assured a further growth. In the field of food irradiation, for instance, the

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

growing attention being given to certain radiation-processed fruits, fish, and meats by large and small companies—in both the food processing and nuclear fields—has been truly significant during the past year or two. It is our feeling in the Atomic Energy Commission (AEC), based on first-hand impressions from speaking to industry representatives, that commercialized radiation processing of one or more products could begin in two to three years, and that from this beginning, a rather slow but steady increase in the number of products thus processed will take place as U. S. Food and Drug Administration clearances for foods are obtained.

Both the AEC and Army must rely heavily on the nuclear industry to help "sell" food irradiation to processors or distributors. Whereas a food industry might be most aware of radiation effects on its specific product, competence for constructing and operating a radiation facility is generally more concentrated in a number of nuclear engineering companies which specialize in this area. The five-year concentrated effort of the AEC in designing and constructing various facilities (*1*), through contracts with several nuclear companies, has been an immeasurable aid both in developing nuclear know-how in private industry and in arousing interest in applied radiation processing. It is the purpose of this presentation to expose the reader to the elements of radiation facility design as experienced from our own history of irradiator development. Pertinent considerations applicable to irradiators are similar to those for conventional, or nonirradiation, facilities.

Let us assume that a food company or processor has general knowledge of the merits of radiation as applied to his product. His interest is sufficiently high that he would like to know specifically how radiation could fit into his operations, the costs and benefits he might expect, and the timing involved in beginning new operations. The problem of "to whom do I turn for help and information?" may in itself provide a stumbling block or be discouraging enough to make the processor think that he should wait another year or so until the picture is clarified.

Usually, however, through only a few inquiries, a good source of information on food irradiation can be tracked down, whether it be one of the several government agencies involved, one of several aggressive nuclear companies actively involved in practical applications, or any of a number of universities or contractors working in the program. (Table I lists AEC contractors currently active in the food-irradiation field.) A hard look must then be given to the practical considerations involved in applying radiation processing.

Costs and Benefits

The next important question is: "what are the anticipated costs and benefits applicable to a specific application?" The food processor unfamiliar with radiation technology may need help in evaluating this question, but

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch009

Table I. AEC Contractors in the Food Irradiation Program

Contractor	*Area*
I. Development	
Massachusetts Institute of Technology	Seafood
U. S. Department of the Interior, Gloucester, Mass.	Seafood
U. S. Department of the Interior, Seattle, Wash.	Seafood
Louisiana State University	Shrimp, oysters
Oregon State University	Seafood
University of Michigan	Fresh-water fish, fruits
U. S. Department of the Interior, Ann Arbor, Mich.	Fresh-water fish
Michigan State University	Fruits
University of California, Davis	Fruits
University of Florida, Gainesville	Fruits
University of Hawaii	Tropical fruits
Arthur D. Little, Inc.	Salmonella
U. S. Department of Agriculture, Savannah, Ga.	Grain and grain products
Hazleton Laboratories, Inc.	Packaging studies and petition preparation
University of Puerto Rico	Tropical fruits
National Bureau of Standards	Electron/x-ray studies
U. S. Department of Commerce	Economics and marketing
Army Pictorial Center, New York	Film production
II. Wholesomeness and Public Health Safety	
University of Massachusetts	Animal feeding
Industrial Bio-Test Laboratories	Animal feeding
AME Associates	Animal feeding, I
AME Associates	Animal feeding, II
Continental Can Co.	Microbiology
U. S. Department of the Interior, Seattle	Microbiology
Massachusetts Institute of Technology	Microbiology
Louisiana State University	Microbiology survey
University of Washington	Microbiology, biochemical
University of California, Davis	Biochemistry of fruits
University of California, Davis	Physiology and microbiology
Cornell University (Geneva Agricultural Experiment Station)	Physiology and biochemistry
U. S. Department of the Interior, Gloucester	Flavor and odor
University of California, Berkeley	Heme proteins
University of Massachusetts	Lipids
Oregon State University, Corvallis	Microbiology
III. Irradiator Design and Construction	
Brookhaven National Laboratory	Radiation engineering
Associated Engineers and Consultants, Garden City, N. Y.	Design, construction of MPDI, Gloucester
Vitro Engineering Co., New York, N. Y.	Design, construction of mobile irradiator and grain products irradiator

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Table I. Continued

Contractor	*Area*
Nuclear Materials and Equipment Corp., Apollo, Pa.	Design, construction of Hawaiian development irradiator; construction of two on-ship irradiators
Processing Equipment Corp., Lodi, N. J.	Construction of one on-ship irradiator; service contract for research irradiators

help is available, directly from AEC, Department of the Army, or the aforementioned nuclear companies (Table I). In most cases, a general estimate of processing costs can be obtained, knowing only the product, the dose required, and throughput per year. A detailed cost estimate—easily done by a nuclear engineering company—must consider several more problems. First, should the radiation source be an isotope (such as cobalt-60), or a machine source, which may generate either high energy electrons or x-rays? Care must be taken to obtain the latest and most accurate costs for either isotopes or machines. Regardless of the source selected, the remainder of the facility will be approximately the same. That is, there may be a need for both pre- and postirradiation cold storage, and there will probably be a need for some type of conveying equipment to transport the product in and out of the radiation area. Ideally, there is an advantage if the radiation step can be tied in directly with the existing processing line.

Another basic question is: "what type of facility would be most applicable?"—i.e., is a fixed or a mobile irradiator most applicable? If a fixed facility is applicable, should it be an in-plant unit or a central facility to be used by several processors? Seasonal availability of a product, near one location, is critical to the economics of radiation processing since typical capital costs for a moderate food irradiation plant may run anywhere between a quarter and two million dollars or more. Where there are relatively short harvest seasons, it would be economically advantageous to plan for irradiation of several products. This, however, requires a more flexible or versatile conveying system past the radiation source and generally less efficient use of the radiation. Thus, while a slight increase in capital cost may be required, the unit cost for processing would be less.

So far, we have looked only at the costs of radiation processing and then only in fairly general terms. One cannot be specific unless specific factors are considered. Those elements most pertinent to determining costs applicable to a given operation include location of harvest area, length of harvest season, product-handling procedures using radiation, type of packaging materials used and package size, atmospheric and temperature control prior to, during, and after irradiation, product production rate, and

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Table II. Cost Estimates for Radiation-

(5-year

	Strawberries (200,000 rad)	
	Capital cost, $	*Operating cost, $*
Capital cost, less source	250,000	50,000
Cobalt-60 at 60¢/curie, 25% efficiency	(200,000 curies) 120,000	24,000
Annual cobalt replenishment (12%)		15,000
Insurance (third party)		20,000
Operating cost (3/shift, 2/shift at $7,000/man, 1/shift at $12,000.		78,000
Facility operation (utilities, etc.)		10,000
Other (misc.)		10,000
Totals	$370,000	$207,000
Throughput	3000 lb./hr. for 3000 hr. year	
Costs	2.6¢/lb.[a]	

[a] Based on idle operation for over ½ year.

total dose required. Their combination and relative importance, together with the normal physiological response pattern of the food involved, will dictate site location, facility size, and ultimate economics.

Some typical rough cost estimates for products probably closest to commercialization are: strawberries 2.6 cents per pound, based on a plant idle for over half of the year; shrimp 1.6 cents per pound; bananas ¾ mill per pound; and sterilized meat 8.5 cents per pound. These costs assume that a cobalt-60 source is used at 25% efficiency and that plant write-off is based on a five-year period. Some of the details of initial capital costs and processing costs, as well as other assumptions, are included in Table II. These are rough estimates, probably not applicable to a specific area or situation. Since the radiation step lends itself to automation, a 20-hour day or 6000-hour processing year is assumed. Finally, unit processing costs will drop as throughput increases. For example, if the throughput of the meat facility in Table II is doubled from 10 to 20 million pounds per year, processing costs would drop from 8.5 to less than 7 cents per pound.

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Processing Selected Products

write-off)

Shrimp (150,000 rad)		*Bananas (35,000 rad)*		*Meat[a] (4.5 mrad)*	
Capital cost, $	*Operating cost, $*	*Capital cost, $*	*Operating cost, $*	*Capital cost, $*	*Operating cost, $*
250,000	50,000	1,000,000	200,000	1,000,000	200,000
(100,000 curies)		(2.4 × 10^{6} curies)		(2.6 × 10^{6} curies)	
60,000	12,000	1,500,000	300,000	1,560,000	310,000
	8,000		190,000		200,000
	20,000		20,000		20,000
	78,000		78,000		78,000
	10,000		20,000		30,000
	10,000		20,000		25,000
$280,000	$188,000	$2,500,000	$828,000	$2,560,000	$863,000
2000 lb./hr. for 6000 hr. year		4 × 10^{6} lb./day for 6000 hr. year		1700 lb./hr. for 6,000[b] hr. year, 8.5¢/lb.	
1.6¢/lb.		0.07¢/lb., or about 3/4 mill/lb.			

[b] Assumes tie-in with existing meat processing facilities.

Benefits gained from the radiation application must overbalance the processing costs. It is easy to generalize on the extension of shelf life and expansion of markets for fish or the reduction in spoilage of certain fruits, or the prolonged storage for months and years at room temperatures for properly packaged meat products. However, the potential radiation processor is interested in benefits as they relate specifically to his case—the higher price per pound he might expect for his product, the marketability, and public acceptance. Except possibly for a consumer educational program to acquaint the public with the wholesomeness and safety of radiation-processed foods, as well as to explain the nature of how the process works, the marketing and economics applicable to a specific processor must necessarily be determined by that processor. Admittedly, the bulk of data amassed on food irradiation has been on a laboratory scale as opposed to a pilot or semiproduction application.

The AEC and Army, however, are now taking several steps to augment this area of effort. First, the AEC, in its low-dose program, has offered to

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the fishing industry use of the Marine Products Development Irradiator in Gloucester, Mass., for experimentation or end-product testing of seafoods. Similarly, upon completion of other large units—the Grain Products Irradiator in Savannah, Ga., the Mobile Fruit Irradiator in California, a small portable irradiator easily moved to any location, and the Hawaiian Demonstration Irradiator in Honolulu, Hawaii—industry participation will be invited for end product testing, demonstrations of proof-of-principle, and possible test marketing. With these factors better determined, a more realistic evaluation of commercialization will be available.

On the sterilization side, the Army has been discussing with AEC the aspects of a cooperative industry-government program involving the construction and operation of a meat-sterilization facility. Although still in the planning stage, a conceivable arrangement could be reached whereby an industry or combination would provide the capital to construct the plant, and the Army would procure a significant percentage of the throughput. AEC participation could include implementation of the program, as well as possible assistance in providing the radiation source. As soon as plans are finalized and it is determined that the project should proceed, a general solicitation of industry interest in participation would be a probable first step.

The practical value of radiation for industrial processing is proving itself in a number of applications such as sterilization of medical supplies, production of chemicals, cross-linking of polymers, and production of semiconductors. Radiation engineering has taken immense strides in characterizing and applying radiation technology. Important factors which are now making radiation more attractive are: (1) source design is adequate to ensure penetration of target material with uniformity; (2) a high portion of the source energy available can be absorbed in the material; (3) proper selection of isotopes or machine energies precludes radioactivity from being produced in the product; (4) reliable control has increased significantly in the past several years, especially in machines; radioisotopes, because they emit constantly, are inherently 100% reliable, but source handling mechanisms are subject to breakdown; (5) radiation sources are now available in quantity at reasonable costs for material, equipment, installation, operation, and maintenance; (6) a safe operation, as evidenced by the enviable record established over the years by the nuclear industry, can be assured.

Conclusions

To sum up the situation as it looks today, these things stand out as particularly impressive:

The use of radiation as a processing tool is growing rapidly. The 70 million dollar business in 1964 practically doubled in 1965 (*2*).

The factors to be considered before using radiation as a food-processing technique are nearly identical to those for a conventional process. A cost-

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benefit analysis or economic analysis for a specific application must be made by the potential processor, taking into account factors peculiar to his individual situation.

There is a large storehouse of information in both food irradiation and radiation engineering. The AEC's Division of Isotopes Development is always available to industry to bring it abreast of the current status of either and to make available names of nuclear companies which can provide detailed assistance.

To translate laboratory data to field conditions, the AEC plans to work with industry in semiproduction test processing.

Finally, the aura of mystery in nuclear energy applications should be dispelled. Just as a food processor need not know how to design and build a refrigerator or freezing unit, neither should he be expected to know how to design or build a radiation unit. In either case, companies and individuals are available to assume this burden for him. However, for a company to remain uninformed of the applications and advantages offered by radiation over conventional techniques will ensure conformity to the old adage, "always a bridesmaid, but never a bride."

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RECEIVED November 9, 1965.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch009

10

Shallow Irradiation of Oranges by Electrons

A Feasibility Study for In-Line Processing

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch010

This paper reviews some experimental data developed to bridge the gap between research and the commercial application of irradiation energy. For convenience these studies were performed with oranges, but the techniques apply to all fruits and indeed spherical shapes where only shallow penetration by electrons is required. The work was designed to generate and present data in a manner which will be meaningful to processers who generally are not expert in irradiation technology. The work was continued to a point where in-line processing could be contemplated. Domestic commercial situations for fruit do not support the construction of a pilot plant at this time.

The interest in irradiation processing of food is widespread, and the tempo of effort, stimulated by real problems, is increasing. A recent international conference (*14*) revealed the volume and diversity of work being carried out around the world. The significant aspect which has not received enough attention is the translation of research results to real commercial situations.

Hardware manufacturers, in the last two or three years, have perfected designs for new electron machines. These units have been designed specifically for continuous in-line processing on a multiple-shift basis. By stressing reliability and flexibility, assemblies which can be maintained by normal production plant personnel are available with full capacity output available for 3000 to 4000 hours without shut-down. The designs incorporate features which permit horizontal or vertical configurations with the most compact shielding design. High output machines, up to 60 kw., are

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In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

available so that converting electrons to x-rays is not only feasible but is copious enough to process high daily throughputs, typical of the food processing industry (*1*).

The ecology, epidemiology, pathology, and microbiology of foods is vastly complex and quite beyond the scope of a hardware manufacturer. Most researchers have limited themselves to studying the influence of irradiation on a variety of foods and their parasitic organisms (*13*). This study from industry is an attempt to bridge the gap between research and commercial application, using some of the new equipment available.

Since this was the initial study in a planned series, it was important to select a relatively straightforward study. This way the program was not bogged down by irresolvable and costly problems generated by the study itself. As noted below, several areas requiring parallel but independent study evolved.

In the United States the interest in irradiating fruits is low, but other areas such as North Africa or the Middle East have a viable commercial interest (*8, 11*). The problem of irradiating spherical shapes uniformly has eluded researchers in the past, and treatment of fruits by electrons has not been carried out on an extensive scale. The selection of oranges then satisfied many aspects in the design of the experiment.

General Considerations

Most of the work performed on fruits and vegetables has been done with penetrating irradiation, generally from isotope sources such as cobalt-60 (*2, 13, 14*). The objective of that work was to extend shelf life by postharvest treatment without using chemicals such as biphenyl, or *o*-phenylphenol (*3, 4, 5*). Unfortunately, using x-rays or γ-rays affects the pulp of the fruit—i. e., breakdowns occur in the acids, sugars, and vitamins, resulting in undesirable flavor.

We then needed to find a way to treat the outer surface of the fruits, where most of the organisms initially propogate in their hosts. In the commercial marketplace the familiar blue-green molds produced by such spores as *Penicillium digitatum* are the problem. A few infected fruits within a case can ruin a shipment under normal conditions in three to four days. Obviously, deep-rooted organisms will not be controlled by the shallow electron treatment.

The special advantages of the electron treatment are control of penetration and dose by beam regulation. Control of penetration implies that only the flavedo (the outer colored skin) need be affected by the radiation energy, thereby avoiding adverse effects. For the orange, there is a thick layer of white pith, the albedo, immediately below the flavedo. By choice of beam energy it is practical to terminate the range of the electrons within this thickness.

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Specific Gravity Measurements

Penetration of electrons in matter is directly proportional to the density of the material. In unit density material (water), penetration at 1 m.e.v. is of the order of 4.6 mm. The last 20% of this penetration is not generally useful since the total energy conveyed to that depth is but a fraction of that distributed at the surface.

However, with oranges, there is no desire to achieve high dose levels other than in the outer skin. The variation in dose through the treated depth will have to be appraised on a pathological basis to see what is significant.

Good quality navel oranges were purchased as the experimental material. The fruits had been distributed by Sunkist Growers, Inc. and were in excellent condition from a consumer's point of view. For the short duration of these tests, the oranges were kept in a polyethylene bag in an ordinary refrigerator. It was assumed that the fruits had received normal post-harvest treatments: washing, waxing, and diphenyl inhibitor treatment.

A few fruits were carefully peeled, and small sections were cut from the peel with a razor. It was practical to separate sections of the flavedo and albedo. Using a standard pycnometer, specific gravity measurements revealed these typical values:

Flavedo only —	sp. gr. 0.867 (22.2°C.)
Albedo only —	0.600 (24.2°C.)
Combination —	0.710 (23.4°C.)

Discussion of Peel

The flavedo of navel oranges is approximately 2 mm. thick. It is fairly uniform, but there is a tendency towards slightly reduced thickness at the poles of the orange (stem and navel). The albedo varies greatly, being thickest at the equator of the fruit and tapering away towards the poles. If a small fruit had been established at the navel, the skin of the main fruit was frequently observed to be open, and the albedo had tapered to nothing, with even the flavedo feathering to reduced thickness at the opening.

Since this lack of uniformity exists, it would seem wise to explore whether or not 2 mm. of penetration is essential. If the energy level is low, then the fruit pulp will not be penetrated at the poles, and uniformity of dose over the whole outer jacket will be an appropriate objective. On the contrary, it may be desirable to orient the fruits preferentially so that the dose reduces slightly from the equator towards each pole; this may be difficult to achieve.

Penetration Data

With a known specific gravity, one can predict the penetration for different thicknesses of material at various energies. The ultimate penetration of electrons with some specific energy is difficult to measure because

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simple detectors may be influenced by secondary effects. However, there are copious data which show what the value may be to within a tolerance of less than 0.20 mm.

Figure 1 shows a projected curve (Curve a) of penetration *vs.* energy. Penetration depth at the 20% level of ionization was selected so that there would be some assurance that samples were not too thick. Curve b shows the test results where penetration was definitely confirmed for specimens of a known thickness.

The test samples were cut from pieces of peel with a razor. It was not easy to produce large flat samples without crushing or deforming the

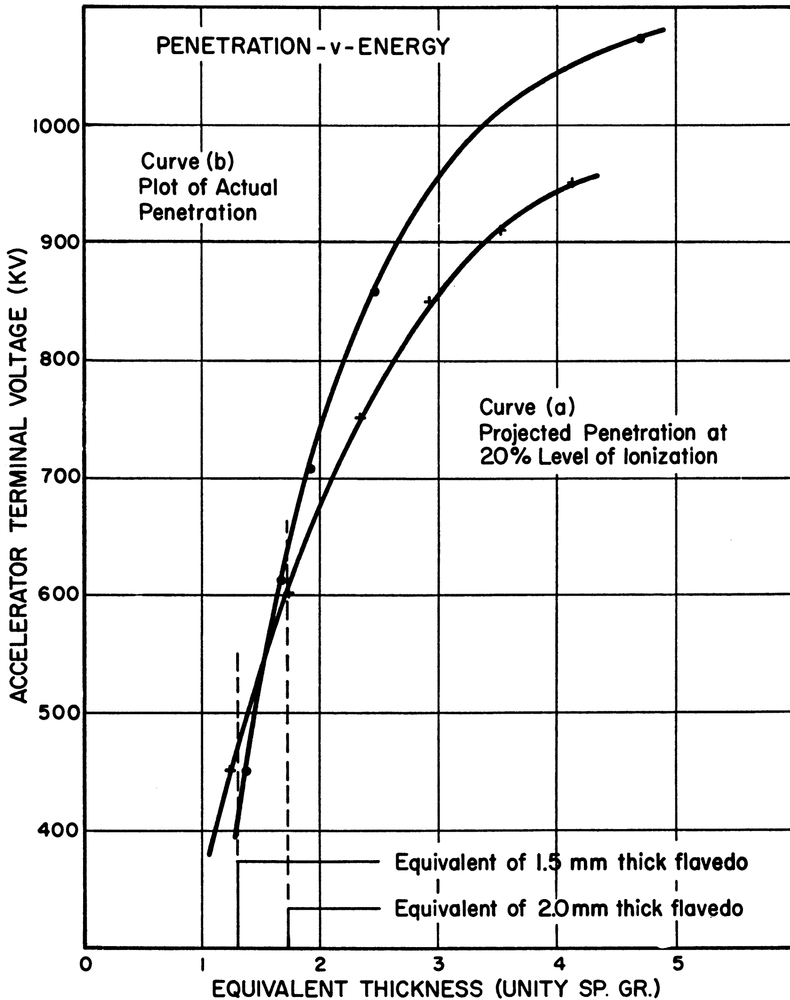

Figure 1. Surface pasteurization of fruits (oranges)

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peel from its natural curved topography. Small samples about 5/8 to 3/4 inches square were therefore used. Each sample was trimmed to reduce the albedo thickness until a certain desired total thickness was obtained. The flavedo was about 2 mm. thick, but it was not measured precisely for each sample. Hence, there is a lack of precision in relating the projected curves to the test curve (Curve b).

It was felt that taking contiguous samples was preferable to constructing models by laminating parts of the skin. The albedo material is spongy and easily crushed, and the samples can be prepared quickly with a minimum loss of moisture, thus retaining more typical characteristics.

The trimmed samples were mounted on standard glass slides with small sections of blue cellophane beneath them. The slides were, in turn, supported about ½ inch below the window of an electron beam unit (ICT). The beam was swept, and the samples were held stationary. Sufficient

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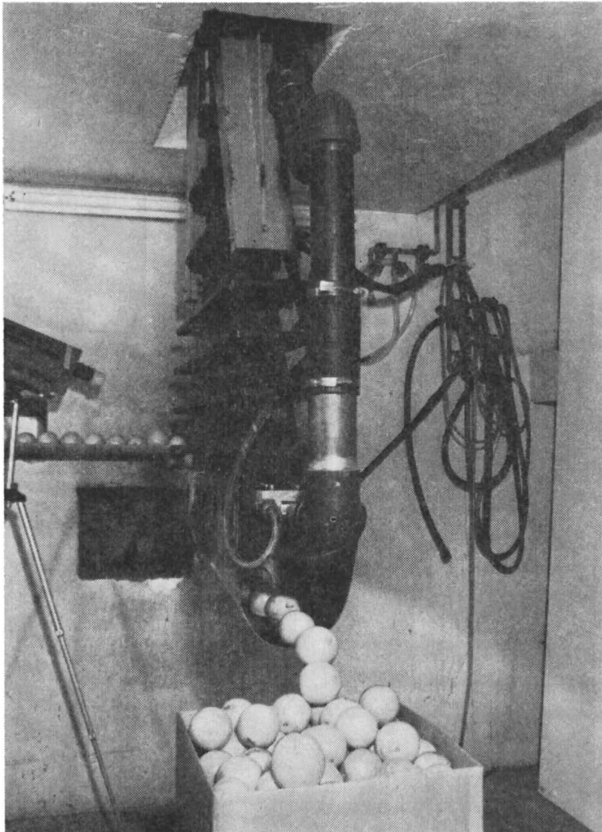

Figure 2. Trial setup for shallow irradiation of fruits (end view). 500 k.e.v. EPS

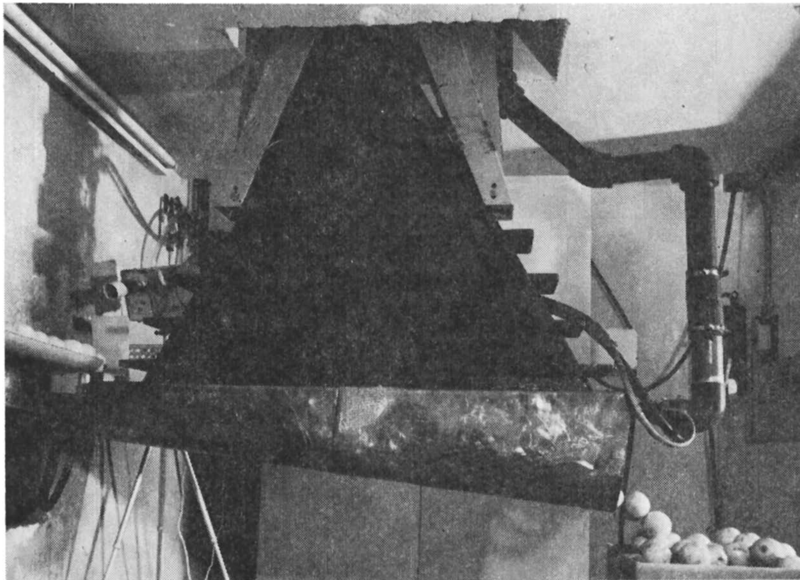

Figure 3. Trial setup for shallow irradiation of fruits (side view). 500 k.e.v. EPS

time was allowed at 1 ma. of beam current to develop a dose of about 4 Mrads at the 20% ionization level.

Cellophane dosimeter strips from beneath the treated samples were then compared with the control piece from the same section of a roll. In all tests, there was clear evidence that penetration had occurred. At the higher energies, there was evidence of secondary effects—backscattering and possibly, secondary emission. The discoloration of the glass slides showing fairly sharp delineation between the uncovered and covered portions gave useful visual evidence of what had occurred.

Experimental Setup

The method described below does not represent the final commercial design for a packing station unit. However, some data are relevant, and a concept for a commercial installation capable of handling 150,000–200,000 fruits per hour is explored.

An existing facility of Electronized Chemicals Corp. was modified to perform the work. The facility comprises an EPS 500 (Electron-beam Processing System) mounted in an accessible vault. The accelerator unit intrudes the central 8 × 10 ft. chamber with its exit window about 4 feet above the floor. On this window flange was mounted a thick lead chute as shown in Figures 2 and 3. The dimensions of the chute and other notes are shown in Figure 4.

A movable tray which supported a sample batch of oranges was mounted normal to the inlet of the chute. When the tray was canted by

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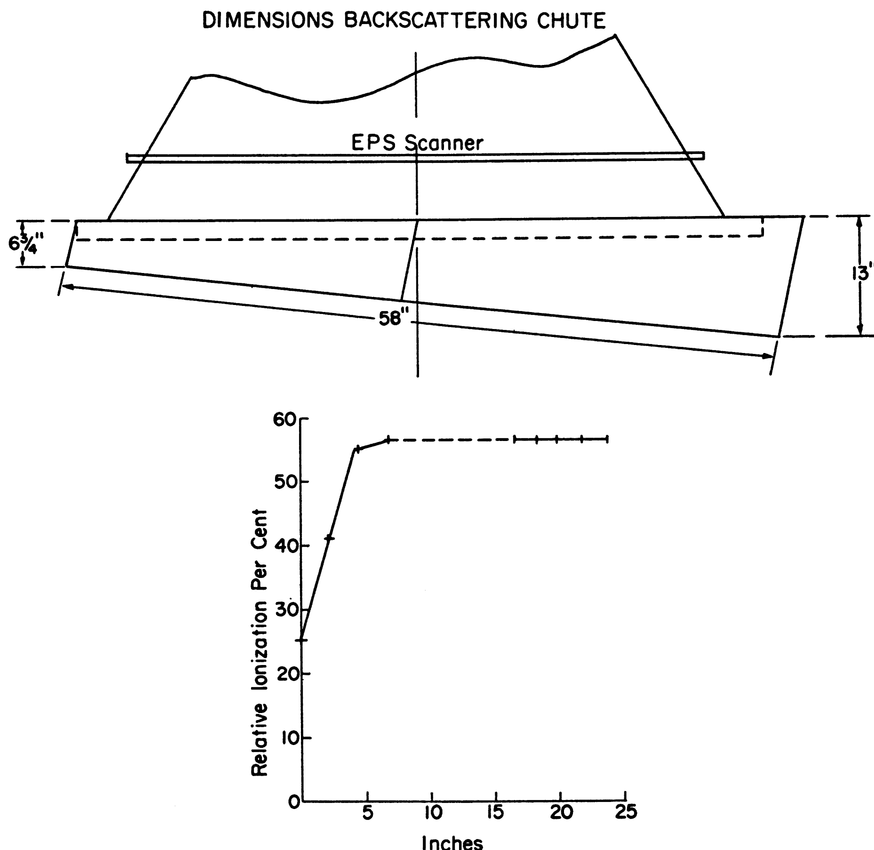

Figure 4. Electron flux distribution

a mechanical system operated from outside the vault, the fruits, actuated by gravity, rolled through the chute. Closed-circuit TV was used to monitor the trials.

The chute was selected because it was the most simple and economical method both to translate and to rotate the fruits. It is, of course, desirable to treat the whole outer skin as uniformly as possible, and with a directional beam system, rotating the product is essential. The slope of the chute was determined from rolling friction considerations, and the traverse time is of the order of two seconds for a typical orange.

Since there are air losses, it is desirable to keep the chute as close to the accelerator exit window as possible. However, by using a metal chute, backscattering can be exploited to take advantage of the electrons' not striking the produce. Backscattering is more effective with materials of high atomic number. For ease of construction and to enhance the effect, lead was used for the chute.

Figure 5 shows a typical cross-section of the electron flux distribution within the chute. This plot was made by appraising the absorbed dose in

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cellophane at various positions after a sheet of this material had been exposed for about 30 seconds under a 2.25-ma. electron beam from a 500-k.e.v. terminal. The smaller figures are percentages of the maximum absorbed dose, and the isobars represent the electron flux within the chute. The diagram is reduced from a full scale cross-section at approximately the midpoint of the assembly.

The flux is slightly asymmetric, and this can be attributed to lack of perfection in shaping the lead. More important is the clear contribution from backscattering which has fashioned the shape of the electron flux so that there is a relatively large area of uniformity. Silhouettes of typical fruit sizes have been indicated so that one can visualize why uniformity can be achieved. Obviously, the pattern at other positions in this variable cross-section chute is different.

The over-all length of the chute, 58 inches, forces each fruit to rotate completely five to six times during its passage. Since the fruits are injected by a gating system normal to the irradiation chute, they already have some rotational energy in this direction. The effect is to add some gyrating motion to the fruits as they roll through the chute. Each element of the surface is then exposed to the variety of flux patterns as it rotates the five to six complete turns to the exit.

Ordinary fruits from retail outlets were used for this work. In triggering a batch through the irradiation chute, the fruits generally ran skin to skin, but the slope was steep enough to produce occasional separations, presumably because of size variations and random effects.

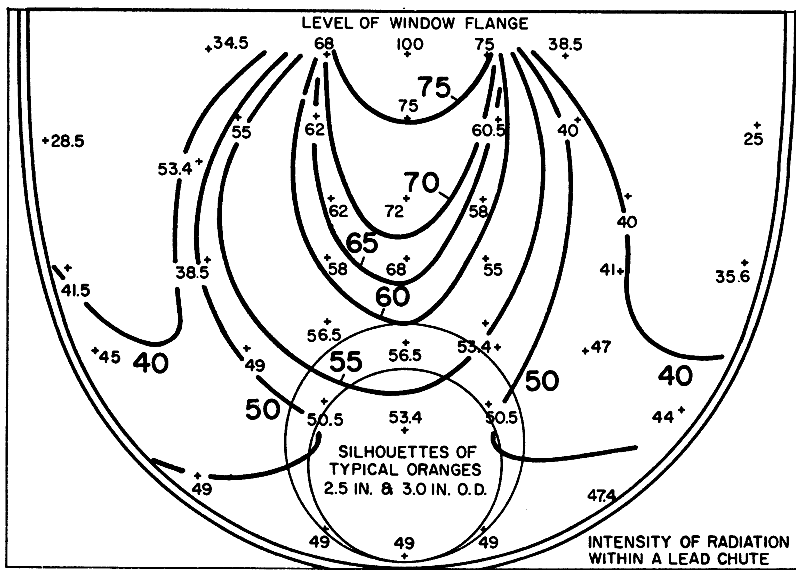

Figure 5. Shallow penetration of fruits

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Uniformity Studies

As discussed below in the section on dosimetry, there appears to be no convenient monitor in the hundreds-of-kilorad range (*9, 10, 12*). Distribution studies were therefore made in the megarad levels with no regard for possible injury to the fruits.

For uniformity, three techniques were used:

(a) An irradiation-sensitive tape—a new proprietary product for which no details are offered

(b) Bleaching of blue cellophane—a relatively well-known technique for the megarad range

(c) Cobalt-silicate glass chips—in anticipation of possible use for correlation in the kilorad range

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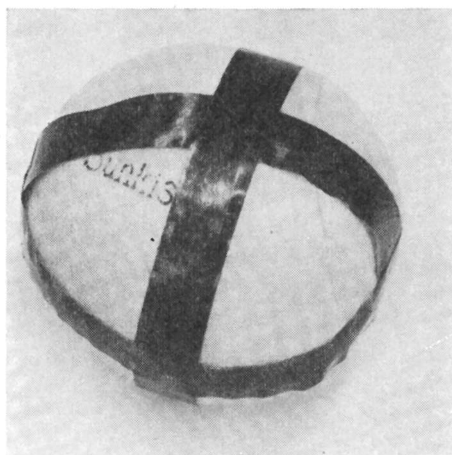

Figure 6. Sample of irradiation-sensitive tape. Note uniformity of color.

Figure 6 shows dramatically the good results achieved with the irradiation-sensitive tape. It is intended for a go/no-go check and has a sharp color change. The indicated dose was of the order of 1.5 Mrad, but more exact data are not available. It is interesting to note that after five days at room temperature no obvious breakdown or other injury could be noted with this sample. To facilitate comparison over the full spherical area, the tapes were stripped from the fruit and laid out for examination.

For the bulk of the work, blue cellophane strips were used. These were secured to individual fruits as meridians at 60° spacings running from stem to navel. After irradiation, each tape was sampled at the stem end, equator, and navel end. Eighteen readings per fruit were sufficient to demonstrate good uniformity. A sample series of readings is shown in Table I.

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Table I. Uniformity of Dose Study[a]

Typical Values *Meridian (60°)*	1	2	3	4	5	6
Stem	1.5	1.5	1.8	1.5	1.25	1.5
Equator	1.5	1.62	1.5	1.5	1.5	1.5
Navel	1.0	1.5	1.5	1.5	1.5	1.5
Stem	1.62	1.62	1.5	1.37	1.37	1.37
Equator	1.62	1.62	1.5	1.5	1.37	1.25
Navel	1.62	1.62	1.5	1.5	1.37	1.25
Stem	1.5	1.5	1.0	1.13	1.25	1.5
Equator	1.25	1.0	1.25	1.25	1.25	1.5
Navel	1.25	1.25	1.13	1.25	1.5	1.25
Anomaly Sample						
Stem	1.37	2.0	2.0	1.8	1.62	1.5
Equator	1.25	2.0	2.0	1.8	1.8	1.8
Navel	1.25	1.8	1.8	1.5	1.8	1.8

[a] Terminal voltage, 500 kv.; beam current, 8 ma.; random rolling

The last group in this table shows the data from one of a few anomalies. It will be noted that the dose indicated for two of the longitudinal strips is lower than the other four. It is hard to explain just how a spheroid can roll under presumed uniform radiation and achieve this kind of exposure. Statistically, these anomalies can be ignored.

Little sampling was performed with the cobalt glass since a limited supply of chips was available. The sampling did, however, confirm uniformity. The techniques of measurement are quite straightforward but require some refinements over the cellophane. The orientation of the chips appears to be quite critical.

Dosimetry

As mentioned above, the cobalt silicate glass chips offer one practical system for measurements in the kilorad range. However, since the chips need to be embedded in the fruit, they are not convenient, and also they are relatively expensive. Nevertheless, these chips may still be the best choice for definitive work at low doses.

It has been argued that scaling the beam current down from a level where acceptable data can be achieved with cellophane is a good approach. This may not be viable if ultimately dosage needs to be measured in 50-krad steps. The precision for controlling beam current is readily available, but this is not necessarily persuasive.

Some liquid systems are presently being developed by the National Bureau of Standards. However, the practical aspects of using such systems on a moving product remain to be explored.

The blue cellophane film has general acceptance and can be conveniently wrapped to the moving product. It is, however, not reliable for

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dose measurements in the low kilorad range. Calibration against current at higher dose appears to be the best present choice (*15*). The scale down-factor, which may be as high as 20, is undesirable but is certainly two orders of magnitude lower than scale factors frequently used in electrical power measurements. Additional work needs to be carried out in this area.

Other Data

Some measurements were taken to appraise the intensity of irradiation along the length of the chute. These are shown in the graph at the bottom of Figure 1. The intensity is more uniform than might be expected. However, the data are incomplete since the whole length was not examined. It was expected that a commercial installation will have quite different geometry, and therefore, its distribution will be more relevant.

Concepts for a Commercial System

The typical throughput for a modern packing station is in the range 120,000–200,000 fruits per hour (*6*). Obviously, a single stream will require the fruits to move at high speeds, a most undesirable arrangement.

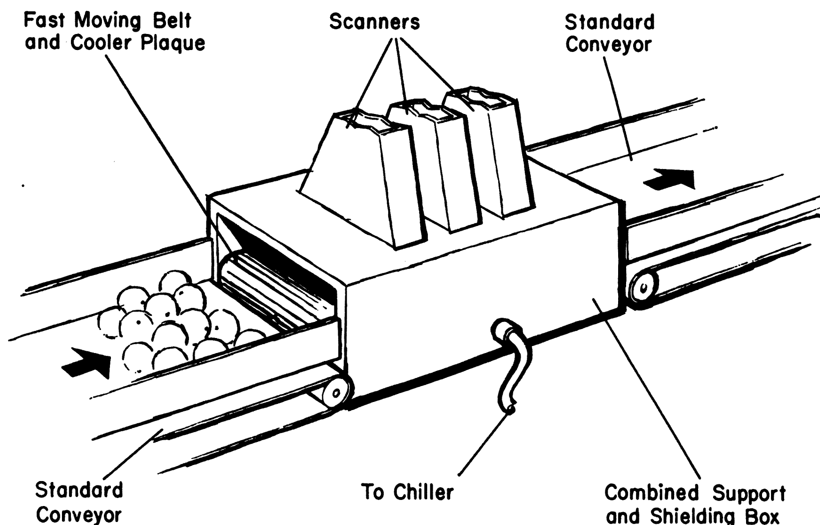

Figure 7. Schematic diagram of fruit irradiation apparatus

By arranging for multiple streams, the through velocity will be reduced. The surface of each fruit should be exposed at least twice to a fairly uniform electron flux. Since the fruits are not perfectly spherical, this will tend to even out the dosage. Further, if there are some nonuniformities owing to lack of beam symmetry or backscattering, these too will tend to be compensated.

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Therefore, multiple accelerator heads should be used to provide a plaque of uniform radiation. The fruits will be carried under these beam ports on a moving metal belt. Stainless steel will be a good choice of materials, and water cooling will be necessary.

If the belt is moving at a higher speed than the through flow (Figure 7), it will contribute to the spinning of the fruits. It is assumed that a smooth belt with appropriate linear speed will not abrade the fruits. Expert knowledge from food handlers will have to be drawn on to complete the details of this conceptual design.

Estimated Throughputs

The trial system handled small batches of fruits at about one-hundredth of the required commercial rate. Since it is only a gravity system, it is not the desired final arrangement.

The EPS unit is rated at 10 kw. accelerator power and is capable of treating some 340 sq. ft./min. to 1 Mrad (31.6 sq. meters/min.). This is empirical data, and allowance has been made for efficiency considerations.

Fruits having 2.5-inch diameter (6.5 cm.) will have an area of 19.6 sq. in. (132 sq. cm.). It is clear that 150,000 fruits per hour is a practical quantity.

Conclusions

The necessary uniformity of dose can be achieved with appropriate backscattering techniques. Dosimetry systems are adequate to continue the program on a realistic basis. More techniques should be perfected. Handling aspects for large quantities of fruits present the major problem (*7*). Cooperation between experienced food machinery builders, users, and the beam specialists should be stimulated.

Acknowledgment

I wish to dedicate this paper to the late Robert J. Van-de-Graaff in memory of his great contribution to both science and humanity.

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RECEIVED November 9, 1965.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch010

11

Cesium-137 Availability and Potential Market

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch011

The fission product and encapsulation plant (FPCE) to be built by Isochem, Inc., in Washington state will produce fully encapsulated fission products for the commercial market. Among these, all of which are extractable from Hanford's plutonium process residues, is cesium-137, a 600-kv. gamma emitter of interest to the process irradiation industry. Isochem will offer cesium in large production quantities and low cost to irradiators of foods, woods, chemicals, etc. Its 30-year half-life promises economies in source array replenishment to compensate for decay. Cesium thus becomes an economic contender for current and planned irradiation applications.

Starting now, and for at least the next 6 years, the firm availability and production capacity of cesium-137 will be greater than the firm demand in this country. I am not speaking of paper availability or of the fictitiously large "potential" demands but of a sincere effort that is underway to have real production capability stay ahead of real firm demand. The current Oak Ridge inventory is capable of meeting all of the firm orders on hand. Continuous production of 1–2 megacuries per year at ORNL will enable it to keep ahead of reasonably foreseeable demand until the large fission product conversion and encapsulation plant (FPCE) at Hanford, Wash., is operational in 1968.

What does this statement mean to the process radiation industry in general and to food irradiation in particular as we approach the point of adding a significant new isotope to the general usage? Most of the research irradiators and demonstration irradiators built so far have used cobalt-60. This was a logical choice since cobalt was readily available commercially in the desired quantity, encapsulation techniques were in use, and basic dosimetry was established. However, it was also generally recognized that the advent of commercial application of food irradiation would require much greater isotope availability and lower irradiation costs. Cesium-137 has long been considered a suitable alternate irradiation source material

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

which would come into general use with the advent of commercial irradiators. Its 660-kv. gamma-ray is sufficiently penetrating for most food irradiators while at the same time being sufficiently soft to be reasonably shielded. Its 30-year half-life promises economies in refueling to compensate for decay. Both of these factors—the reduced shielding necessary and the longer lifetime—lead to lower irradiation cost. Perhaps the greatest promise of cesium-137 lies in the hope of greatly reduced initial radiation cost. Since cesium-137 is a fission product and therefore available as a true by-product of any fission reaction (*2*), it might well be possible to recover it from reactor waste streams in great quantity and at low cost.

We have now embarked on a program to realize this potential. The U. S. Rubber Co. and Martin-Marietta Corp. have created a new, jointly-owned subsidiary—Isochem, Inc. This new company will build a fission product conversion and encapsulation plant at the Hanford, Wash., reservation to produce fully encapsulated fission products for commercial use (*5*). The plant is designed with four separate production lines, each for a different fission product. The capacity of each line varies with the process involved and the batch sizes and processing time. The capacity of the single line normally used for cesium-137 has been set at 29 million curies per year to meet the projected market demands of the early 1970's (*1*). At these production quantities, cesium-137 should be available at less than ten cents per curie for large irradiators.

Food irradiation has entered a new era since the basic feasibility of its technology was established. FDA clearance has been granted on some foods. On other foods, research work, data gathering, and testing have been completed, and the petitions for clearance are under review. We are entering the phase of pilot commercialization, calling on the market analysts, economists, and public relations people to join the food technologists in preparing the marketplace. The design engineers are striving for the next step in sophistication and for the design simplicity that will bring lower costs with improved reliability. It is at this point that cesium-137 should be factored into the industry's growth plan.

The Atomic Energy Commission (AEC), which has contributed so greatly to food irradiation, recently announced plans to reduce the price of cesium-137 (*3*) to a point where the initial cost would be equivalent to cobalt-60 on the basis of radiated power—i.e., assuming that a 4:1 ratio of radiated power gives a Co:Cs price ratio of 4:1 in cents per curie. Different specific designs and variations in encapsulation cost may result in differences in the initial total source cost. It is, however, hoped that a long-term economic review would favor cesium because of the advantages of longer half-life—e.g., lower operating cost, reduced replenishment problems, time operating efficiency, reduced calibration problems, etc. The AEC has also agreed to make cesium-137 available from government facilities until it is available commercially. Isochem is preparing for that future growth by

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch011

investing heavily in production facilities. These two positive steps will allow the industry to plan ahead with assurance. The designer will be free to use whichever energy source can give him the best design. The commercial operator will be assured of long-life radiation sources and the continuing availability of large quantities of cesium from commercial producers (*4*).

It will require approximately 3 years to complete the design, construction, check-out, and licensing of the fission product plant. The AEC action in making available its production facilities in the interim provides continuous availability, which, starting now, will more than adequately serve the growing needs of the industry. Assurances of adequate quantity and reasonable prices, while fundamental to planning, are not the complete answer. Chemical compounds, physical properties, and source shapes will be studied to optimize radiation efficiency, cladding integrity, and source flexibility. Further economies can result by standardizing source plaques as this becomes feasible.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch011

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RECEIVED November 9, 1966.

12

Radiation Pasteurization of Fish and Shellfish

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch012

Irradiation is a practical means of preserving fish and shellfish and is sound from the standpoint of food stability and economy. With practical application comes the responsibility for protecting the consumer and producer by adequate quality control measures, which will vary with the type of fish or shellfish and its major components. Perhaps the most important quality control measure which can be used is the selection of wholesome products prior to irradiation. Irradiation will not improve the quality of poor food, but it will retain the attributes of good food.

The irradiation of fishery products covers a broad area and includes microbiological, biochemical, organoleptic, and engineering problems in the field of fishery technology. Many advances have been made in recent years in the radiation pasteurization of fish and shellfish.

Thirteen species of fish have been studied, and optimum dose level and postirradiation shelf life have been established for them. Most of the major species of fish are suitable for irradiation, and the conditions for commercial application have been ascertained (*15*). The preservation of shrimp and oysters by radiation pasteurization has been shown to be both feasible and successful by chemical, bacteriological, and organoleptic studies of the tested products (*11*). Several field organoleptic trials on public acceptance of gamma-irradiated shrimp and oysters were conducted by the Department of Food Science and Technology at the Louisiana State University, in both coastal and inland cities, and at Fort Lee, Va. Panels from all walks of life preferred the irradiated samples to their nonirradiated counterparts (Tables I and II). Ronsivalli (*14*) reported that the acceptability of irradiated haddock fillets was determined by U. S. Army troops at Fort Lee, Va., according to a test designed by the Quartermaster Research and Engineering Field Evaluation Agency. This agency, which made a statistical analysis of the results, reported that over 400 men involved in the taste test could not differentiate between irradiated 30-day-old haddock

In Radiation Preservation of Foods; Josephson, E., el al.;
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Table I. Storage-Life Range of Radiation-Pasteurized Fish and Shellfish of the Pacific Coast at Tentative Recommended Levels of Radiation

Product	*Package*	*Dose, Megarad*	*Storage Temp., °F.*	*Non-irradiated, Days*	*Irradiated, Days*
King crab meat	Can	0.2	33	5–14	28–42
			42	3–7	14
Dungeness crab meat	Can	0.2	33		
			33	6–11	28–42
			42	2–5	14–21
Petral sole fillet	Vacuum-packed in cans	0.2 and 0.3	33	4–10	28–42
			42	4–7	14–17
Pacific halibut	Vacuum-packed in cans	0.2	33	4–9	21–42
			42	4–5	14–28

Table II. Organoleptic Scores on Nonirradiated and Irradiated (0.20 Mrad) Ice-Stored, Shucked Oysters[a]

Sample Treatment	*Score After Listed Storage Period*			
	Initial	*6 days*	*12 days*	*18 days*
Irradiated	9.4	8.7	8.0	6.3
Nonirradiated	9.7	8.0	6.0	3.8

[a] Ratings are averages for 15 individuals. Values are averages for participants on taste panel for attributes of odor, appearance, sweetness, flavor, and texture.
Code of scores. 10. No change from fresh product of highest quality.
8. First noticeable slight change in attributes.
6. Moderate degree of changed attribute: increased in intensity and occurrence from score of 8.
4. Definite or strong degree of changed attribute.
2. Extreme degree of changed attribute.

Table III. Storage Life Range at 33° and 42°F. of Irradiated King Crab and Dungeness Crab Meats, Petrale Sole Fillets, and Pacific Halibut

Radiation Dose, megarad	*King Crab Meat*		*Dungeness Crab Meat*		*Petrale Sole Fillets*		*Pacific Halibut*	
	33°F.	*42°F.*	*33°F.*	*42°F.*	*33°F.*	*42°F.*	*33°F.*	*42°F.*
	Storage Life, Days							
0	5–14	3–7	7	2–5	4–10	4–7	4–9	4–5
0.1	21	7–14	21–35	14–21	—	—	—	—
0.2	28–42	14	28–42	14–21	28–35	14–17	21–42	14–28
0.3	42	14–21	49–56	21–28	35–49	14–21	42–56	—
0.4	49	28	56	35	35–42	14–28	42–56	35
0.6	49	35	—	—	42	21	42–49	42

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch012

fillets and top quality fresh frozen haddock fillets. Miyauchi (*9*), working on the storage life and acceptability studies on irradiated Pacific crab and flounder, reported that radiation-pasteurized crab meat and petrale sole fillets (Table III), stored as long as 4 weeks at 33°F., received a higher rating by a large majority (from 78–96%) of the judges as compared with non-irradiated controls. On the basis of the results obtained by him, Miyauchi has made the following recommendations for the successful preservation of Pacific crab meat.

Pacific crab meat (Dungeness and king crab) and flounder fillets should be irradiated at about 0.2 megarad, which appears to be the minimum dose that will give a reasonable shelf life extension at a storage temperature of 33°F. (Table IV). The advantages of using this low level of irradiation are: (*a*) it allows the survival of microorganisms capable of producing recognizable evidence of spoilage in the product at the end of its storage life; (*b*) bacterial spoilage probably will occur if the product is mishandled, such as holding the product at elevated temperatures, making the product unfit to eat; (*c*) bacterial spoilage will occur before degradation from other causes in appearance, odor, flavor, and texture become a major problem; (*d*) and, all-important, the cost of irradiation per pound of product would be kept at a minimum.

The radiation-pasteurized products must be stored at the lower above-freezing temperature range (32°–36°F.) to derive maximum benefits of the radiation pasteurization process and to assure the safety of the product (Table IV).

Table IV. Typical Shelf Life Extensions

Product	*Dose, Rads*	*Normal Shelf Life, Days*	*Irradiated Shelf Life, Days*
Shrimp	150,000	14–21	21–40
Crab	200,000	7	35
Haddock	200,000	12–14	30
Clams	400,000	5	30

Microbiology of Irradiated Fish and Shellfish

The success of preserving many perishable foods by radiation pasteurization or sterilization is primarily attributed to the destruction of microorganisms which accelerate food spoilage. The storage life of many foods cannot be extended by radiation doses which result in sterilization because artifacts produced by these high levels are detrimental to the flavor and odor of the foods. Consequently, the lower pasteurization doses used often result in the survival of certain microorganisms which resist radiation or which may recover from radiation effects upon storage in a suitable environment. Kelner *et al.* (*4*) reported that the physiological state of the organisms at irradiation determines to some extent their sensitivity to radiation and that under specific conditions a certain bacterial population can

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recover from the damaging effects of ionizing radiation. Dollar (*1*) has summarized the basic microbiological problems in radiation as follows:

Changes in the natural microflora of sea foods as a result of irradiation and during subsequent storage at low temperature.

Sensitivity of various indicator bacteria and food poisoning organisms to irradiation in relation to growth phase and suspending medium.

Effect of pasteurizing irradiation on the competitive growth (repression) activities of the natural microflora of sea foods.

Spoilage Microflora. In irradiated crab meat at 0.2 Mrad, nonmotile gram negative bacteria predominate soon after irradiation, a few yeasts may appear, and cocci generally disappear. However, after several weeks' storage gram-positive rods tend again to become important in the populations. It is significant that there is no recrudescence of a normal pseudomonad spoilage microflora in stored irradiated crab meat, and that the distribution of radiation resistance levels remains relatively constant among the population surviving radiation throughout its outgrowth.

Bacteria of Public Health Significance. STAPHYLOCOCCI. The sensitivity of staphylococci to radiation depends partly on the suspending medium. It is greatest in late lag and early lag growth phases and least in the stationary phase. No outgrowth of staphylococci in irradiated or unirradiated sea foods at temperature below 12°C. was observed. Above this temperature, outgrowth occurred, more readily in irradiated than unirradiated material.

SALMONELLA. Different *Salmonella* species were found to vary widely in radiation sensitivity. Salmonellae surviving irradiation were able to grow on sea foods incubated at 22°C. but actually declined in number on sea foods held at 11° and 5°C.

CLOSTRIDIUM PERFRINGENS. Spores of types A and B were resistant to radiation at low levels. Surviving *Cl. perfringens* grew well in irradiated crab meat held at 22°C. but declined in number when held at 11° or 8°C.

ENTEROCOCCI. These organisms are moderately resistant to radiation and will grow in crab meat held at 11° and 22°C. but not in crab meat held at 1°C.

Competitive Repression of Growth. *S. aureus* grows well in sterilized but poorly in nonsterile crab meat. This is apparently caused by competitive repression of *Staphylococci* growth by the normal spoilage microflora. Crab meat exposed to pasteurizing doses of radiation permits *Staphylococcus* outgrowth at higher temperature (22°C.). If spoilage is allowed to proceed to a fairly advanced stage before irradiating the crab meat, radiation does not reverse the inhibitory effect on *Staphylococci*. This indicated either the exhaustion of an essential nutrient by the spoilage microflora or the production of an inhibitory substance.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch012

Table V. Radiation Effect upon Optimum Incubation Temperature, Chromogenesis, and Utilization of Glucose of Resistant Organisms

Bacteria		*Temp., °C.*	*Chromogenesis*	*Utilization of Glucose*	
				Acid	*Gas*
S. flava	UI[a]	37	Yellow		
	R	25	White		
A. eurydice	UI	25			
	R	10			
B. firmus	UI	25	White	+	−
	R	10	Pink	−	−
B. pumilus	UI			+	−
	R			−	−

[a] R designates irradiated culture; UI, unirradiated.

The reports on radiation-resistant organisms on other sea foods include codfish (*8*), flounder, rockfish, and salmon (*5*), and haddock and clams (*10*). Liuzzo and co-workers (*7*) studied the morphology and metabolism of seven radiation-resistant bacteria isolated from fresh Gulf shrimp to ascertain if radiation altered their characteristics. They observed radiation-induced differences in optimum incubation temperature, chromogenesis, carbohydrate and vitamin utilization, and action on litmus milk (Tables V and VI).

Generally the bacterial count in a sample of food determines the quality of the sample. However, the number of bacteria present does not necessarily indicate the condition of the food. Before making a microbiological appraisal of the quality of foods, survival of pathogenic bacteria after irradiation of foods must be considered. Many radiation-resistant bacteria do not contribute to rapid decomposition of foods, but several species can hasten spoilage by their metabolic activities. Members of the genera *Bacillus, Clostridium,* and *Micrococcus* belong to this latter group. For example, work being done by Novak and co-workers (*13*) has shown that *Bacillus laterospores,* a radiation-resistant bacterium in oysters, will cause formation of indole in the oysters. Indole is an end product of protein

Table VI. Differences Induced by Radiation on Culture Action on Litmus Milk[a]

Bacteria		*Reaction*[b]	*Curd*	*Peptonization*	*Reduction*
B. pumilus	UI[c]	n (7)	+ (2)	+ (4)	− (7)
	R	a (4)	+ (7)	+ (7)	+ (7)
B. megaterium	UI	n (7)	+ (4)	+ (7)	− (7)
	R	n (7)	− (7)	+ (7)	− (7)

[a] Number of days required for action shown in parentheses.
[b] Reactions classed as neutral (n) or acid (a).
[c] R designates irradiated culture; UI, unirradiated.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch012

degradation; therefore, this bacterium appears to be involved in proteolytic activities in oysters during ice storage. Investigations by Novak with the cooperation of the Alabama Department of Public Health (*12*) showed a significant decrease in the number of coliform organisms in oysters irradiated at 0.3 Mrad and stored in ice. After 3 days' postirradiation, the irradiated oysters showed a plate count of 30 with a coliform count and an *E. coli* count (EC) of less than 2. The nonirradiated oysters showed a bacterial count of 1500 with a coliform count of 1000 and an EC of 130. After 10 days' postirradiation, the irradiated oysters showed a plate count of 460, with a coliform count and EC of 27. The nonirradiated oysters showed a bacterial count of 29,500 with a coliform count of 16,200 and an EC of 980 (Table VII).

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch012

Table VII. Destruction of Coliform Organisms in Oysters by Gamma Irradiation at 0.3 Mrad

	Nonirradiated			*Irradiated*		
Days Post-irradiation	*Total plate count*	*Coliform count*	*E. coli count*	*Total plate count*	*Coliform count*	*E. coli count*
3	1,500	1,000	130	30	2	2
10	29,500	16,200	980	460	27	27

The bacterial counts show extreme variations in irradiated fish or shellfish irradiated at 0.2–0.3 Mrad after 14–21 days storage at 33°F. These variations can range from no bacteria from one sample irradiated at 0.2 to 0.3 Mrad to millions of bacteria per gram on another sample irradiated at the same levels of radiation. To control these variations, it will ultimately be necessary to understand some of the factors that contribute to radiation resistance.

Biochemistry of Irradiated Fish and Shellfish

The biochemical effects of radiation on fish and shellfish can be classified under the following headings.

Radiation Biochemistry of Carbohydrates. Normal autolytic processes are not affected by pasteurizing doses of ionizing radiation. The principal source of volatile base in irradiated and unirradiated or sterile fish or shellfish tissues is through enzymatic amination of adenosine monophosphate to the flavor-active compound inosine monophosphate.

Irradiation of model systems containing glucose and amino acids at concentrations similar to those found in fish muscle extracts has shown that only the glucose is damaged significantly. Such damage reduces the amount of glucose available for oxidation by *Psuedomas* bacteria isolated from fish tissues. Reactive oxides of nitrogen are formed in significant concentrations when solutions and major phases containing air or nitrogen are subjected to low doses of irradiation (*2*).

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Radiation Biochemistry of Proteins and Amino Acids. Up to the levels of 500 krads the changes in pigment properties of heme proteins are similar to those caused by oxidation or reduction. Changes in the proteins, in addition to deamination and loss of —SH groups, indicate a partial denaturation similar to that induced by hydrogen ions. This results in some enhancement of reactivity of the heme portion (*2*).

Radiation Biochemistry of Flavor and Odor. Some 17 carbonyl compounds have been isolated in irradiated fish, and 12 have been identified. Nonirradiated clams show an increase in carbonyls during storage. Following irradiation at 450 krads the concentration of carbonyls increases sharply. The use of cryogenic temperatures significantly lowers the production of carbonyls. (*2*).

Radiation Biochemistry of Lipids. The rancidity that develops in stored fish is the result of oxidative reactions. Work being done with methyl docosahexaenoate shows that irradiation per se does not cause observable or measurable changes in this long-chain fatty acid ester. However, changes involving fatty acids go on at a higher rate in irradiated fishery products than in the nonirradiated counterparts (*16*).

Radiation Biochemistry of Vitamins. Liuzzo and co-workers (*6*) working on the stability of B vitamins in Gulf Coast oysters preserved by gamma irradiation have reported that thiamine, niacin, pyridoxine, biotin, and vitamin B_{12} were partially destroyed by irradiation. Thiamine and pyridoxine were the most seriously affected. Riboflavin, pantothenic, and folic acid showed increases, probably owing to an additional release of the bound vitamins which made them more available to the assay organisms (Table VIII). The vitamin assays were conducted microbiologically on two separate coast oysters obtained several months apart and irradiated at 0.2 to 0.6 Mrad with gamma irradiation from a cobalt-60 source.

Radiation-Induced Changes in Shrimp Enzymes Involved in Spoilage

The effects of low-dose gamma irradiation with cobalt-60 on the iced storage life extension of fresh shrimp were investigated by Grodner (*3, 13*). Doses of 0.15 to 0.2 Mrad administered to brown shrimp (*Peneaus aztecus*) prior to storage in crushed ice significantly reduced the degree and amount of melanosis and resulted in a retention of the desirable organoleptic attributes of the product (*3, 13*).

Studies on irradiated solutions of phenolase showed that the enzyme was inactivated. The radiation-induced changes were found to be different from those reported to occur upon denaturation of proteins. Infrared absorption spectra revealed that deamination had occurred. Acid- and base-binding groups were reduced in number rather than increased, and the optical rotation became more dextrorotatory than levorotatory. It was fur-

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Table VIII. Differences Induced by Radiation in Culture Utilization of Certain B-Vitamins[a]

Vitamin	Amount per Assay Tube, μgrams	Culture Growth as % Transmission					
		B. minutiferula		*G. homari*		*B. megaterium*	
		UI	*R*	*UI*	*R*	*UI*	*R*
Niacin	0.05	69	100				
	0.1	73	100				
	0.2	68	100				
	0.3	56	100				
Pantothenic acid	0.01	49	100				
	0.03	52	100				
	0.05	45	100				
	0.1	42	100				
	0.2	40	100				
	0.5	42	100				
Thiamine, mμgrams	10			64	100	100	79
	20			53	100	100	80
	30			52	100	100	85
	50			50	100	100	90

[a] Values are averages of two assays determined in duplicate. R designates irradiated culture; UI, unirradiated.

ther shown that the copper of the enzyme was not oxidized by a dose of 0.2 Mrad.

The presence of oxygen was shown to enhance inactivation of the enzyme, while the presence of ovalbumin in the phenolase solution protected the activity. This was accepted as evidence that indirect action of the radiation was responsible for the inactivation of phenolase since the presence of dissolved oxygen or protein would have no effect on direct collisions of the γ-rays and the enzyme molecules.

The liberation of sulfhydryl groups was not detected after irradiation of 0.5% ovalbumin solutions. Since it was shown that the presence of inert protein protected, rather than inactivated, phenolase, it was concluded that thiol groups, produced by disruption of disulfide bonds in the protein, had no significant role in phenolase inactivation.

Irradiation of the substrate, 3,4-dihydroxyphenylalanine, accelerated the reaction. Therefore the alteration of substrate by irradiation was not found to be responsible for the decrease in melanosis observed in fresh shrimp.

Reports indicated that the phenolase responsible for melanogenesis of shrimp was located in the shell and concentrated primarily at the joints between segments. It was concluded that water and oxygen may diffuse through these joints, and that their radiolysis products were responsible for the inactivation of phenolase and subsequent reduction of melanosis.

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Irradiation of commercial shrimp in which melanogenesis had begun accelerated the reaction. It was postulated that the quinone-like end products of the reaction sequence acted as competitive inhibitors for the reaction and also functioned as radiation protectors for the enzyme. Subsequent irradiation would destroy the capacity of the end products to inhibit the reaction and simultaneously preserve the enzyme activity, thus accelerating further melanogenesis.

It was ultimately concluded that low-dose gamma irradiation of fresh shrimp reduced melanosis and resulted in a significant extension of their iced-storage life. However, if shrimp were held beyond the onset of melanogenesis, subsequent low-dose irradiation accelerated blackspot formation, which reduced consumer appeal, and therefore was definitely undesirable (*3*, *13*).

Radiation Studies with Oysters

Irradiation of oysters was studied by Novak and co-workers (*12*) at the Department of Food Science and Technology, Louisiana State University.

Oysters for these studies were collected from beds near New Orleans and transported to a nearby commercial packing house. They were ice-stored overnight and professionally shucked the following morning. The facilities of this commercial packing plant enabled us to pack the oysters according to present FDA regulations. The oysters were washed in running tap water for 2 minutes and allowed to drain for 5 minutes. All draining was on FDA-approved stainless steel skimmers, which had perforations of at least 1/4-inch diameter located not more than 1 1/4 inches apart. The oysters were distributed evenly over the draining surface of the skimmer but were not otherwise agitated during the draining period.

After washing and draining, the oysters were packed in 1-pint cans, placed in Arctic hampers, covered with ice, and transported to the LSU Nuclear Science Center. Here, they were divided in half; one part served as the nonirradiated control, and the other part was subjected to gamma irradiation doses ranging from 0.2 to 0.3 Mrad. The irradiation was performed within 24 hours after the oysters came from the beds. After irradiation, both the irradiated and the nonirradiated controls were stored in crushed ice, which was added as needed.

Samples were withdrawn after 1, 2, and 3 weeks for chemical, bacteriological, and organoleptic tests. Results typical of the public organoleptic ratings are shown in Table II.

Table IX. Tentative Levels of Radiation for Shrimp and Oysters

Product	*Package*	*Dose*	*Poststorage Conditions, °F.*	*Shelf Life (Control), Days*	*Total Shelf Life, Days*
Shrimp	Sealed in Mylar bags	0.15 to 0.2 mrad	33	14–16	21–28
Oysters	Pint cans	0.2 mrad	33	14–16	20–21

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch012

The radiation dose which was judged optimum for preserving oysters was 0.2 Mrad. Levels beyond this point resulted in the production of a pale yellow exudate from the oysters, which lowered their acceptability for the attribute of appearance.

The tentative levels of irradiation for the successful preservation of shrimp and oysters now recommended are summarized in Table IX.

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13

Fruit and Dry Product Irradiation Processes

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch013

The tolerance limitation of fruit for irradiation establishes the maximum acceptable dose. If this dose controls decay organisms, the use of irradiation for a particular fruit may appear promising. Response to irradiation may be influenced by fruit maturity, variety, pre- and postharvest temperatures, handling, and extent of fungus growth. Climacteric fruits irradiated prior to the normal rapid increase in respiration usually show an immediate increase in respiration and the production of ethylene. These fruits are frequently retarded in ripening.

Approximately 30 billion pounds of fruit are sold each year in the United States, and about half is consumed fresh. There are various estimates of spoilage losses resulting from decay in marketing fresh fruits. A fair estimate for strawberry losses appears to be 15%, even with improved handling practices.

Many investigators are seeking further improvements in the handling of fresh produce. Among methods receiving much attention are the use of postharvest chemicals, retardants of senescence, thermal treatment, controlled atmospheres, new packaging techniques, better temperature control in storage and transit, and irradiation. One method may work well with one fruit or a particular variety of fruit but not with another fruit.

A reduction in the amount of fruit spoilage during marketing is urgent, but treatments designed to reduce spoilage must not unduly alter the natural quality of the fresh fruit. The use of ionizing radiation is one method of reducing spoilage. High doses of irradiation will completely sterilize or kill microorganisms, but flavor, texture, and appearance of the fruit will be atypical. Therefore, low dose pasteurization levels must be used if gross changes within the fruit are to be avoided.

Several summary reports and reviews on fruit irradiation are available (*1, 2, 3, 4, 5, 6, 7, 8*).

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

Factors Influencing Fruit Response to Irradiation

Any statement with regard to the response of fruit to irradiation needs to include the condition of the fruit prior to irradiation. The physiological status and disease condition will have a marked influence on this response.

Species and Fruit Maturity. Great differences exist among fruit species. In general, fruit can be classified as exhibiting or not exhibiting a climacteric increase in the rate of respiration and ethylene production as they ripen. Peaches, pears, mangoes, and tomatoes are typical fruits in the climacteric class. If they are irradiated before climacteric, the normal physiological development is modified. Usually there is an immediate increase in respiration and the production of ethylene, and ripening is frequently retarded. If the fruits are irradiated during the climacteric, there is little change from the normal respiratory response.

Citrus fruits are representative of the nonclimacteric class. Following harvest, the rate of respiration of citrus slowly declines. Irradiation does not noticeably influence ripening changes in these fruits.

Variety. Frequently extreme differences are found in the response of species or varieties to irradiation. Irradiated Southland peaches tend to become somewhat mealy following irradiation, but the Loring and Dixieland varieties do not. Valencia oranges show considerable peel injury from irradiation but not Pineapple oranges. However, this could be more of a seasonal response than a varietal difference.

Temperature of Fruit. Several conditions will cause physiological stress in fruit. Each fruit species varies as to the minimum temperature it can tolerate. Fruits exposed to a temperature below the minimum tolerance prior to or after harvest will show symptoms of chilling injury. Irradiation also creates physiological stresses, which are often cumulative, so that symptoms may become evident at a higher minimum temperature. For example, untreated grapefruit can be stored at 55°F. without any apparent chilling injury as observed on the peel, but after 200-krad irradiation or storage at 55°F., typical symptoms of chilling injury appear.

Fruit Handling. The methods of handling fruit following harvest will influence the response to irradiation. Many fruits are highly perishable. They are easily damaged by rough handling, undergo rapid physiological changes and deterioration, and if contaminated with decay organisms may spoil in a short time. Fruits should be precooled soon after harvest and irradiated as quickly as possible.

Fungus Growth. Surface contamination of fruits is usually controlled by fungicidal materials as well as ionizing radiations. The greatest problem of spoilage control is experienced when spores have contaminated cuts and wounds and the fungus has grown within the tissues. Ionizing radiations penetrate deeply and exert an effect within fruit tissues, where chemicals cannot go. However, the more extensive the growth of the fungus

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch013

within the tissues, the higher the radiation dose required for inactivation. If spoilage control requires a large dose, the tolerance of the fruit may be exceeded.

Radiation and Physiological Effects on Fruits

The tolerance limitation of fruit for irradiation establishes the maximum acceptable dose. If this dose is sufficient to control decay organisms, the use of irradiation for the particular fruit may appear promising.

Irradiation has a serious effect on softening and texture changes of many fruits. The level required for disease control in grapes is too high to avoid extreme softening of the tissues. Therefore, irradiation does not appear possible for grapes.

Off-flavors and atypical odors have been developed in some fruits, particularly tangerines and limes.

Increased development of red color in the flesh of peaches and nectarines has been noted by a number of workers, but color development has been delayed in tomatoes, pears, and mangoes.

Fruits Showing Promise for Radiation

Whether it will be economically feasible to irradiate particular fruits will depend upon a number of factors. A most essential point is that the fruit must not undergo appreciable quality change with irradiation levels necessary for destruction of the disease-causing organisms.

Strawberries appear to be most promising for commercial radiation. There is little change in texture, flavor, color, and odor after a 200-krad dose, and the treated fruits have stood up well in test shipments. Berries are produced in large quantities in concentrated areas for several months.

Peaches are quite susceptible to brown rot, caused by the fungus *Monilinia fructicola* (Wint.) Honey. If the infection is not too severe, it can be controlled at about 200 krads. There appears to be a marked variation in varietal response. Some varieties retain relatively good quality while others show gross changes, particularly in texture. The anthocyanin pigment content in the flesh increases as a result of irradiation. Nectarines show a response to irradiation similar to that of peaches.

The ripening process in pears is inhibited by irradiation. This should be advantageous since overripening of pears is frequently a problem.

Oranges have shown variable results between locations. When grown in the more arid regions, no peel injury of irradiated fruit was observed. However, Valencia oranges produced in Florida, under humid conditions, showed considerable peel injury with treatment. With 200 krads or lower treatment, the flavor of the juice was as acceptable as that from untreated fruit. At this irradiation level, decay in Valencia oranges was significantly reduced.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch013

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Irradiation of Dry Products

Irradiation appears to hold some promise for treatment of dry products. Insects and their larvae can be controlled in dried fruits, such as figs and apricots.

Dehydrated irradiated vegetables will absorb water much more rapidly than the nonirradiated product. This process may have great promise for use in manufactured products, such as dried soups, where rapid reconstitution is highly desirable.

Consumers must be educated to accept irradiated produce. Irradiators adapted to rapid fruit treatment must be designed. Costs will largely depend upon the volume of commodities treated.

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14

U. S. Army Radiation Laboratory

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch014

The world's largest irradiation laboratory for preserving foods by ionizing energy is operated by the U. S. Army at Natick, Mass. It has two radiation sources, a food development-preparation laboratory, and an experimental development kitchen. The sources are cobalt-60 and an electron accelerator with a maximum energy of 24 m.e.v. They are supported by three laboratories: health physics, radiochemistry, and dosimetry. Other facilities include a section which develops suitable containers for use in irradiation experiments. By housing all these facilities in one location, one has complete control over each phase of the experimental procedure. The Army is concentrating its efforts on meat sterilization to provide the armed services with food which needs no refrigeration and yet resembles fresh food when prepared.

In 1962 the U. S. Army opened at its Natick Laboratories in Natick, Mass., the world's largest irradiation laboratory (*2*) for preserving foods by ionizing energy (Figure 1). This laboratory is unique in that, in addition to having two radiation sources, a 24-m.e.v., 18-kw. electron linear accelerator and a 1,250,000-curie cobalt-60 isotope source, it includes a food development–preparation laboratory and an experimental development kitchen (Figure 2).

Before construction of this laboratory the Army depended upon industrial and national laboratories to provide irradiation services on a contractual basis. This procedure lacked the complete control over experiments that is obtained if all functions are housed in one facility, where scientists of the various disciplines can freely discuss experimental designs and results.

The food irradiation program in the United States is broad in scope and divided principally between the U. S. Atomic Energy Commission (AEC) and the U. S. Army, with the Army's concentrating on meat sterilization and the AEC's concentrating on fruits, vegetables, and fish pasteurization. Sterilization and pasteurization are differentiated by both the

In Radiation Preservation of Foods; Josephson, E., el al.;
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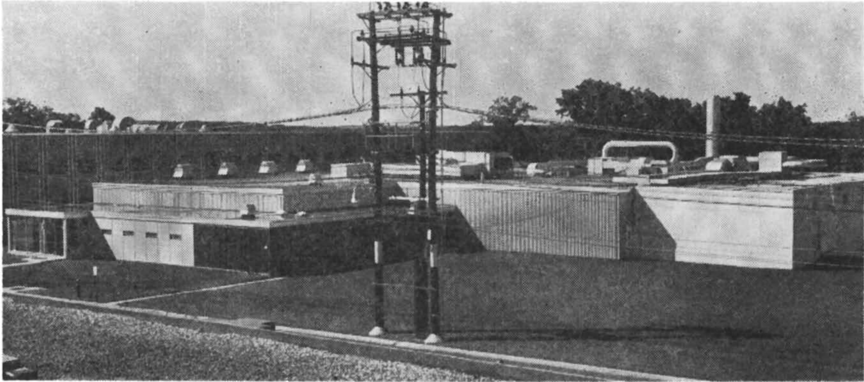

Figure 1. Exterior of the U. S. Army's radiation laboratory

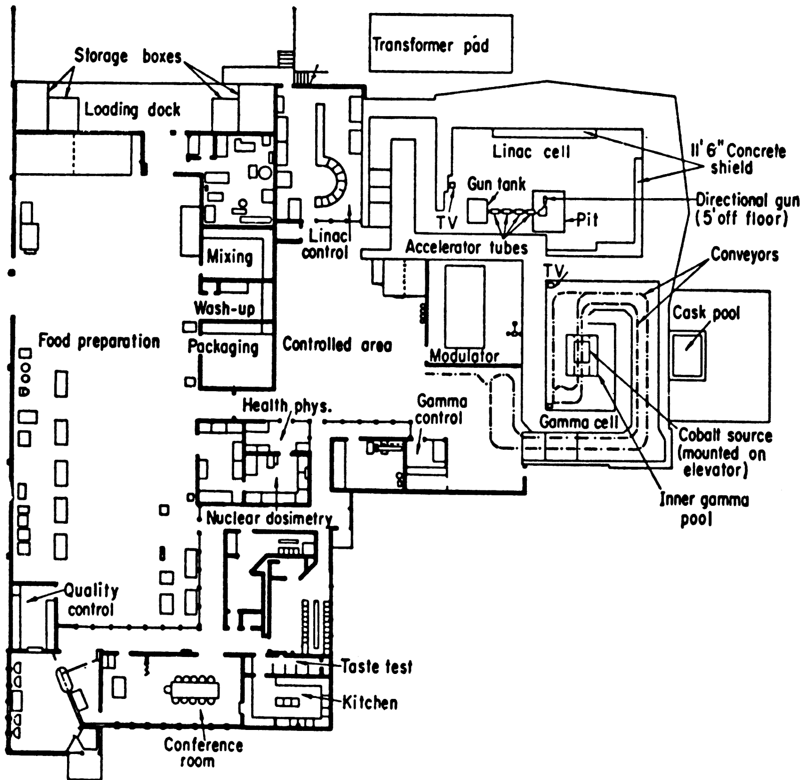

Figure 2. Floor plan of the U. S. Army's radiation laboratory

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch014

level of the radiation dose employed and the shelf storage life of the products. The sterilization program of the Army has as its goal to supply the armed services with foods which do not require refrigeration during storage yet resemble fresh foods when prepared.

Radiation Sources

The radiation field was surveyed for all possible types of sources that could be used for food sterilization. Since cobalt-60 and electron accelerators with energies greater than 5 m.e.v. showed the most promise, they were chosen.

Electron Accelerator Facility. The linear accelerator facility consists of an electron accelerator with a maximum energy of 24 m.e.v. and peak power of 19.2 kw. at this energy (Table I). This machine is located in an irradiation cell 48 feet long by 30 feet wide (Figure 3). Other components of the accelerator facility are the power supply in the modulator room and the control console and electronic shop in the control room. The irradiation cell is entered by passing over a man-trap and through a labyrinth (5 feet 6 inches wide), constructed of solid concrete blocks stabilized with grout to facilitate modifications of the labyrinth or to enable larger pieces of the machine to be removed or modified if necessary. The electron machine is located near the east wall to permit various conveyor configurations and to provide experimental setup room. The electron machine is currently

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Table I. Beam Parameters of NLABS S Band Linac

Microwave frequency, mc.	2856
No. of accelerator sections	4
Klystrons (1 per section)	4
Maximum peak r-f power, Mw.	5
Maximum average r-f power, kw.	10
Energy range, m.e.v.	3 to 30
Beam energy at maximum efficiency, m.e.v.	22
Peak beam current at maximum efficiency, ma.	375
Rated average power, kw.	
At 2 m.e.v.	0.68
At 6 m.e.v.	1.38
At 12 m.e.v.	6.48
At 18 m.e.v.	12.6
At 24 m.e.v.	19.2
Maximum peak beam power, kw.	19.8
Maximum peak beam current, ma.	375
Normal operation duty factor	0.002
Beam pulse lengths, μsec.	0.5 to 5.5
Pulse repetition rates per second at 0.5 to 5 μsec. (Single pulses available)	15, 30, 60, 90, 180, 360
Energy spectrum	2.5% at half current for 20 m.e.v.
Spot size	0.5 cm. diameter min.

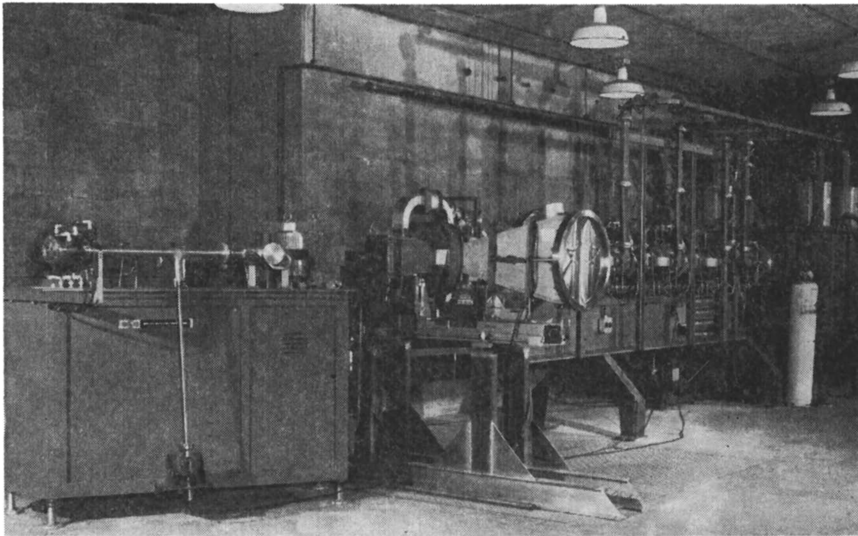

Figure 3. The electron linear accelerator with energy analyzing and scanning systems

equipped with a beam-handling system that includes three windows—straight ahead, 30° right, and 30° left—as well as a 90° window with a scanning bucket (16 x 3 inches) to permit irradiation of larger samples than the nominal electron beam spot size of 1 cm. For most food irradiations, samples are conveyed past the 90° window at a controlled rate. The conveyor used is an overhead monorail type with each carrier self-propelled through the labyrinth by a small low voltage d.c. motor. At approximately 3 feet from the center line of the scanner the carrier is coupled to a servochain drive that controls speed through the beam. This drive system has a range from 0.0004 to 0.8 foot per second and is controlled by a servosystem with less than 1% variation.

In addition to the present energy-analyzing system, the accelerator is being equipped with a beam-handling system consisting of two pairs of quadrapole magnets, a servoslit system, and two 45° bending magnets which will allow control of the spot size and shape. This modification will improve the machine's usefulness for basic research in the radiation chemistry of foods, dosimetry systems, and chemical systems at various dose rates. To provide adequate biological shielding when converting the electron beam to x-rays, the cell wall directly in front of the straight-ahead port and the 90° left port are 11½ feet of standard concrete with 2 feet of solid concrete blocks stacked within the cell. This wall tapers to a minimum of 8 feet in the corners of the cell. This design was chosen for maximum safety and economy.

Owing to the possible production of radioactive isotopes, ozone, and oxides of nitrogen, the accelerator cell is equipped with an independent

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ventilation system which normally removes approximately 3000 standard cu. feet per minute of air, corresponding to 10 air changes per hour when the machine is not in operation. This air is drawn into the cell, through the labyrinth, by the air-exhaust fan located on the roof of the building. This fan has a capacity of 9000 scfm and is used at capacity when the machine is operating. For this higher rate, 6000 scfm of fresh air is supplied at the entrance of the labyrinth by a make-up fan. The air exhaust is filtered through a roughing filter and an absolute filter to remove any radioactive particles before the air is exhausted up a 20 foot stack to the atmosphere. The top of this stack is approximately 38 feet above the ground.

MODULATOR ROOM. The power supplies, pulse-forming network, and r.f. drivers for the accelerator are all located in a steel vault in the modulator room. This room is provided with access ports and a 1-foot square sand-filled trough to permit cables to be installed between the Linac cell and the modulator room and between the cobalt cell and the modulator room. In addition, ports are provided, through which an Omniscope may be inserted to observe operations within the cells during operation.

CONTROL ROOM. The accelerator control room was designed to enable the operator to view the entrance to the cell directly from the control console as a safety precaution (Figure 4). From this console an operator can

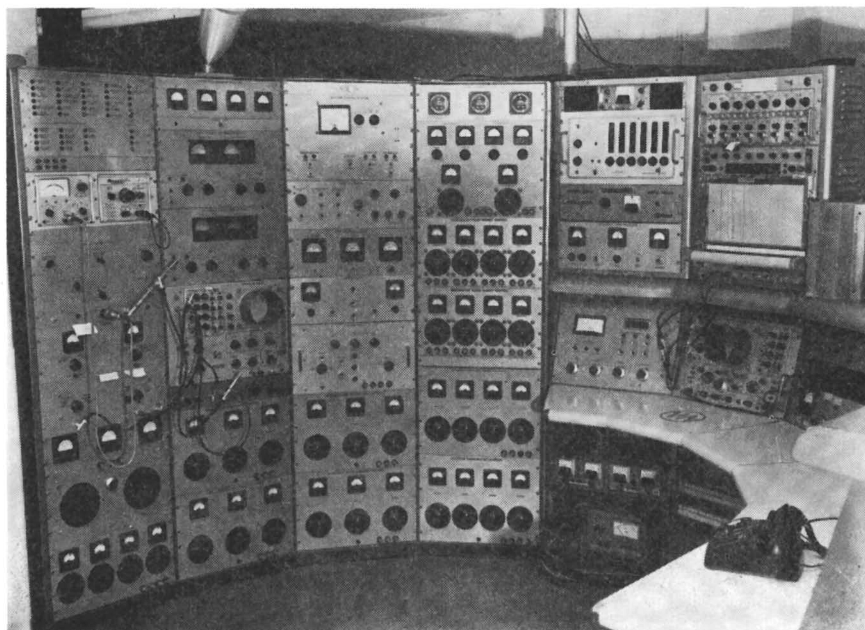

Figure 4. The accelerator control console

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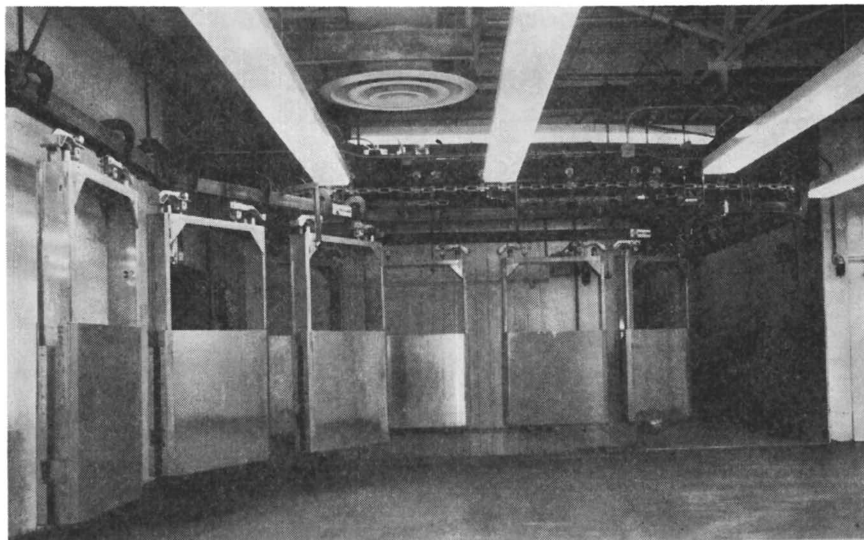

Figure 5. The cobalt cell entrance and carrier loading area. The carriers shown are of bulk type with internal dimensions of 41 inches wide × 33½ inches high and 7½ inches deep

control all the operations, including tuning the machine, advancing the carriers, regulating the conveyor speed, and controlling the cell lockup.

This control room also houses work benches and test equipment used to maintain and modify the machine.

The electrical cables between the control room, the modulator room, and the irradiation cell are in pipes below the floor. A cable trough is being installed in the labyrinth to increase the cable capacity between the cell and the control room.

Cobalt Facility. The cobalt facility consists of the cobalt-60 source located in the irradiation cell; source loading pool, outside the building; source elevator pool, in the irradiation cell; and control console, in the cobalt control room.

IRRADIATION CELL. The irradiation cell is entered by passing over a man-trap and through a labyrinth (7.0 feet wide) constructed of standard concrete (Figure 5). The cell walls provide a minimum of 6-foot shielding in all directions to provide adequate biological shielding for 3 megacuries of cobalt-60. The initial loading of the cell was approximately 1.25 megacuries.

The cobalt-60 source consists of 392 individually encapsulated rods (*1*). Each cobalt rod, which is 0.725 inch in diameter, is covered by a stainless steel jacket with welded end closures. This, in turn, is covered with an aluminum jacket (Figure 6). The doubly sealed slugs are 0.943 inch in diameter and approximately 10.7 inches long. The cobalt rods were encapsulated before activation in 1245 aluminum alloy to minimize activation

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of impurities in the aluminum. The rods may be arranged in various geometries by modifying the elevator source jig plates. For all work to date the rods have been arranged in two parallel plaques (Figure 7), each consisting of 49 vertical source holder tubes mounted on a mandrel, at-

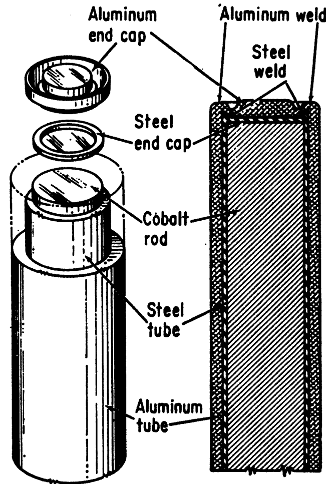

Figure 6. Exploded and cross-section view of an encapsulated cobalt rod

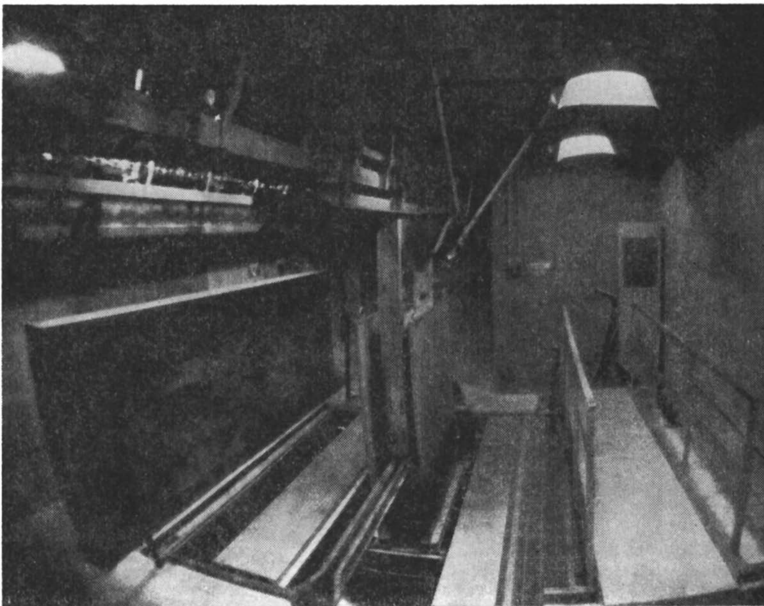

Figure 7. Interior of cobalt cell as seen through the omniscope. A carrier is in the dwell position with the source raised

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tached to the jig plate. Each source holder tube contains four cobalt-60 rods positioned on top of one another.

The over-all dimensions of the active portion of the plaques are 42.2 inches high by 56.2 inches wide and the center line distance between the two plaques is 16 inches. The original center line distance between the two plaques of 11 inches was changed to 16 inches to improve the over-all dose uniformity in the carriers.

CARRIERS. Samples to be irradiated are arranged in a carrier and transported into the irradiation cell through the labyrinth and in turn between the source plaques by an overhead monorail conveyor, which has a variable-speed drive capable of conveyor speeds from 0.6 to 60 feet per minute. The carrier selected depends upon the temperature requirements and size of the samples to be irradiated. For samples larger than a No. 3 (can) cylinder (4¼ x 7 inches) a bulk-type carrier is used, 41 inches wide, 33½ inches high, and 7½ inches deep (inside dimensions) (Figure 5). For microbiological samples a special carrier has been constructed which can handle up to 44 10-mm. test tubes with temperature control between —190° and +100° ± 20°C. (Figure 8). For samples up to a No. 3 cylinder a special low temperature carrier has been constructed of four stainless steel Dewars (inside diameter 4½ inches) which can accommodate 20 No. 3 cylinders (Figure 9). It can be used to control the sample temperature between room temperature and —190° ± 15°C.

For temperature control runs it is necessary to run the conveyor in the dwell mode of operation—i.e., the carrier is transported to a fixed location, centered between the plaques in the cell, at which point a liquid nitrogen line is remotely attached to the carrier (Figure 10). The rate of nitrogen flow, hence the cooling rate, is controlled by copper-constantan thermocouples in the carriers which are connected to temperature controllers outside the irradiation cell.

For normal operation the conveyor is operated in a batch-type operation. In this mode the carriers are transported at 60 feet per minute, to minimize the transport time, to a fixed position between the source plaques. The carrier is disconnected from the power chain and remains in this position for a predetermined period of time to obtain the desired radiation dose. It is possible to perform this operation with the source either raised from the water or in the lowered position.

In a second mode, the carriers are transported to a fixed position at one end of the source at the maximum speed. The conveyor drive shifts to a selected speed and continues to convey the carrier past the source. When the carrier is completely past the source, the conveyor shifts back to the maximum speed and transports the carrier from the cell.

In a third mode, the carriers are attached to the conveyor drive chain at fixed intervals and are conveyed through the labyrinth and between source plaques at a fixed speed. This mode is the most time-con-

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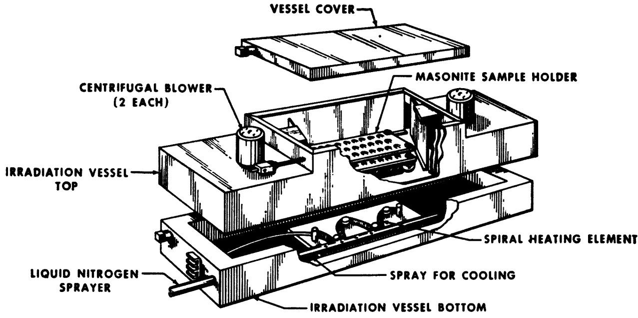

Figure 8. The cryogenic biological radiation box used for irradiating samples in test tubes at controlled temperature between 80° and −196°C.

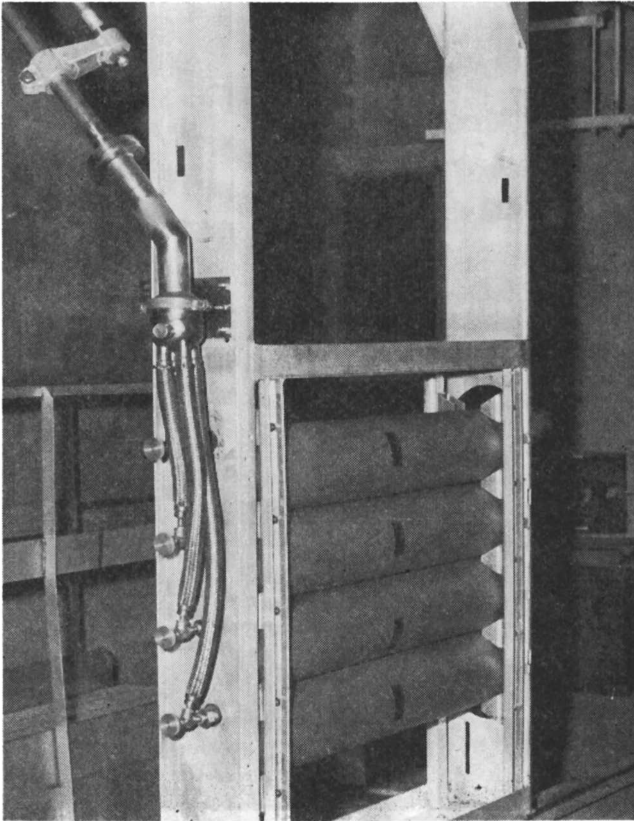

Figure 9. Low temperature carrier designed to accommodate 20 No. 3 cylinders. For irradiations at controlled temperatures between room temperature and −190°C.

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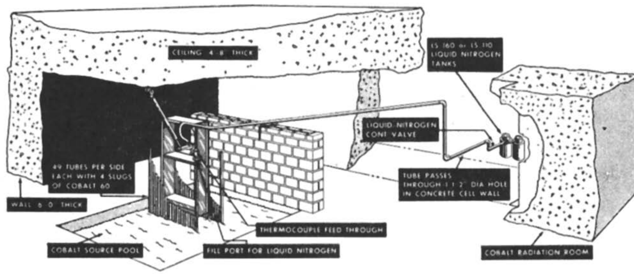

Figure 10. Perspective view of cobalt cell with liquid nitrogen cooling system

suming and has been used only for irradiating large volumes of fruits at low total doses.

The conveyor used with the cobalt source was originally designed to be used with no lubrication. However, because of rust buildup on the wearing surfaces it was found to function better if lubricated (Atomic Energy of Canada, Ltd., SKF-M-3 ball grease base).

The cobalt source is raised into the irradiation cell from the bottom of its storage pool for irradiation by the source elevator. The drive for the elevator is located in the modulator room. The motor for lifting the source is mounted on the main drive shaft by a torque-sensitive drive mechanism. This mechanism provides a safety feature by which, if the source jams, the elevator motor will hit limit switches and stop.

The cobalt-60 is received in stainless steel lead-lined casks (approximately 18 tons) which are lowered into the receiving pool by portable

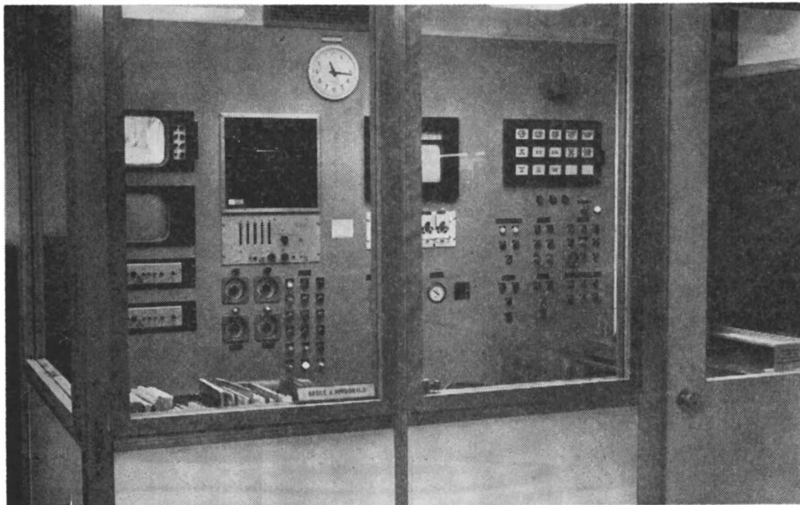

Figure 11. The cobalt-60 source control room

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch014

cranes. The cobalt is removed from the casks remotely under 23 feet of water by using long-handled tools, and it is then transferred into the source storage pool in the cell, where it is arranged in source tubes and loaded on the source elevator for use.

CONTROL ROOM. The cobalt control room was designed to enable the operator to observe directly the carrier loading area and the entrance to the cobalt cell and to view remotely the interior of the cell by use of television cameras (Figure 11). From the control room the operator can control the conveyor functions and the source elevator in addition to monitoring air flow, water activity, and pool water level.

Laboratory Support

The radiation sources are supported directly by three laboratories: a health physics laboratory, a radiochemistry-low level counting laboratory, and a dosimetry laboratory.

Health Physics Laboratory. The health physics laboratory is located at the entrance to the radiation sources controlled area (Figure 2). From this laboratory the access to the radiation sources area is monitored and controlled. The health physics laboratory is equipped with portable beta, gamma, and neutron survey meters of various designs and ranges to facilitate the area monitoring, air monitors for airborne contamination, and anticontamination equipment. It is equipped with monitors and alarms for the area radiation detectors, pool water level indicators, and access doors. It also has ready access to the counting equipment of the radiochemistry laboratory.

Low Level Counting Radiochemistry Laboratory. The low level counting-radiochemistry laboratory consists of an area of approximately 200 sq. feet. This laboratory is used for measuring the level of radioactivity in foods. This activity can originate from naturally occurring radioisotopes, fallout from nuclear devices, or induced activity resulting from the radiation sterilization process. It has been demonstrated that no measurable activity occurs as a result of irradiating food with gamma-rays from cobalt-60 and cesium-137, with x-rays of energies less than 5 m.e.v., or with electrons with energies less than 10 m.e.v. To perform these measurements, the laboratory is equipped with RIDL-400 and 1600 channel analyzers with accessories, a Beckman Wide Beta II counter, a Packard Tricarb counter, a Tracerlab Low Beta counter, two 5 x 5-inch and two 3 x 3-inch NaI detectors, and conventional end-window Geiger counters. The shield for the 5 x 5-inch NaI crystals was fabricated from the mid-section of a 16-inch 1918 naval gun barrel (Figure 12). This shield was selected because it was cast before detonation of nuclear devices and is, therefore, free of fallout.

Dosimetry Laboratory. The radiation dosimetry laboratory is adjacent to the health physics laboratory and measures approximately 190 sq. feet, with an additional 300 sq. feet in the controlled area. All source

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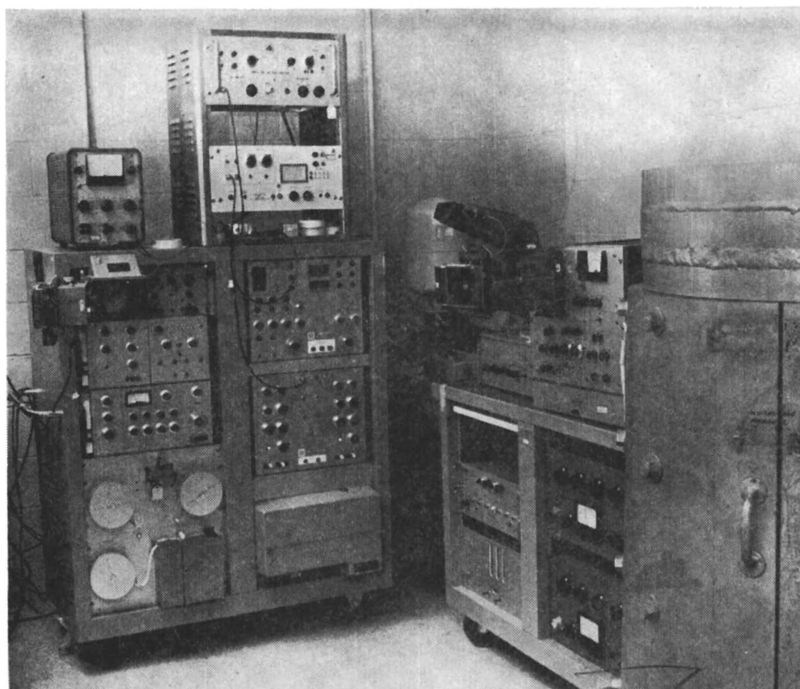

Figure 12. Low level counting equipment consisting of two RIDL multi-channel analyzers with accessories and a modified gun barrel shield

calibration is performed in these areas. This dosimetry consists of measuring the transmittance change in solutions, films, and glasses, measuring the e.m.f. or pH change in solutions, and measuring the electrical properties of materials and gas production as a function of the radiation dose. This laboratory is equipped with three spectrophotometers, a gas chromotograph, an automatic titrator, and standard analytical instruments.

To calibrate the cobalt source, three systems are most often used: ferrous sulfate, ferrous sulfate–cupric sulfate, and ceric sulfate. Dosimeters of these solutions are prepared by filling 5-ml. chemical-resistant glass ampoules with approximately 5 ml. of solution and flame-sealing the ampoules. The ampoules are then arranged in "phantoms" of Masonite or similar materials (Figure 13) to simulate the food items. These phantoms are placed in containers similar to those used for food products, and arranged in the conveyor carrier in which they are transported into the irradiation cell. Because of the upper dose limit of the ferrous sulfate and ferrous sulfate–cupric sulfate dosimeters (40,000 and 800,000 rads, respectively), these systems can be used only to establish the dose rate in the facility and not to monitor the total dose during food irradiation. The ceric dosimeter which

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is suitable for measuring megarad doses is used for quality control during processing.

For calibrating the accelerator, poly(vinyl chloride) films and a simple water calorimeter are used in addition to monitoring and controlling the electrical parameters of the accelerator which affect the dose output and, in turn, the absorbed dose. The poly(vinyl chloride) is used primarily for establishing the depth dose in samples irradiated with the scanned electron beam. This film is relatively thin when compared with the range of 10

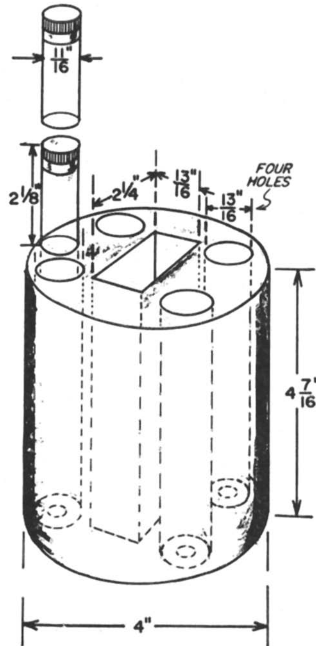

Figure 13. Phantom constructed of 1/8-inch masonite disks to simulate foods canned in a No. 2½ can. Dosimeters shown are made of polystyrene ampules

m.e.v. electrons and is suitable for measuring the dose in the range of 0.5 to 3.5×10^6 rads. The film changes color when irradiated, and this color change is stabilized by annealing the irradiated film for 15 minutes at 64°C. The dose received is determined by measuring this color change at a fixed wavelength (3950 A.) in a spectrophotometer. This film is also used to establish the beam spot size and location and to indicate that the samples have been irradiated.

The water calorimeter is used as a production monitor. It consists of a water-filled plastic Petri dish, containing a temperature-sensing device

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch014

(normally a copper-constantan thermocouple). The Petri dish is thermally insulated with approximately 2 inches of Styrofoam insulation. The dose received by the calorimeter is determined by measuring the temperature change in the calorimeter caused by irradiation. This calorimeter is reproducible within ±2% and has a calculated accuracy of ±2%. It is used for production monitoring by irradiating a calorimeter before and after the sample carriers containing the product to confirm that no changes occurred during the processing.

Product Development

The food preparation area of approximately 6000 sq. feet is fully equipped with steam kettles, rotisserie, grills, deep fat fryers, retort, ovens, smoke house, blast freezer, and storage boxes (Figure 14).

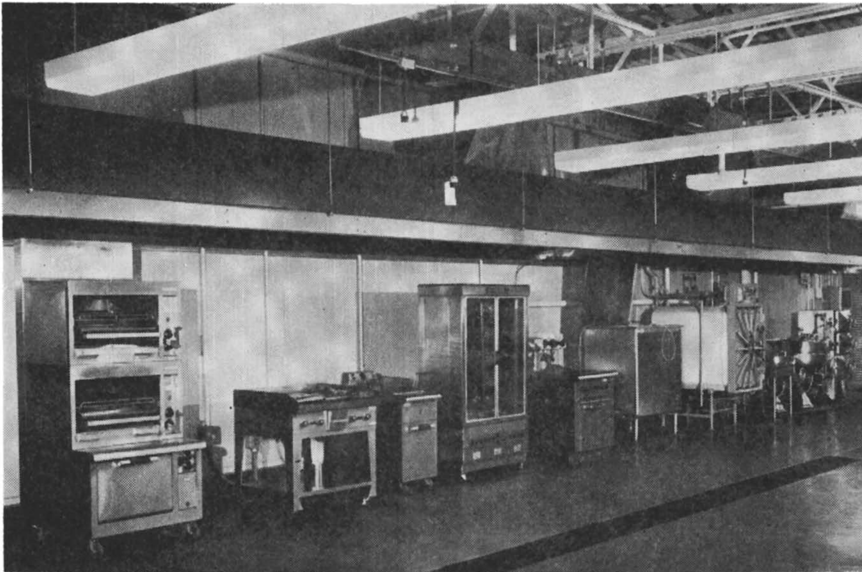

Figure 14. Food preparation area

Container Development

The Container Division at NLABS provides technical assistance in developing containers suitable for use in irradiation processing. The use of the electron accelerator has placed the most stringent requirements upon packaging. These requirements are dictated by the range limitation of 10 m.e.v. electrons, which is 3.3. cm. for a single-side irradiation. This range can be increased by modifying the accelerator's method of irradiating and the product conveyor system. Because of this range limitation it has been

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necessary to develop a flexible package which is impermeable to gases, light, easy to seal, inert to the foods which it contacts, and not appreciably affected by radiation. A series of films has been explored in detail for use.

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RECEIVED November 26, 1966.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch014

15

Amino Acid Destruction in Beef by High Energy Electron Beam Irradiation

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Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch015

When ground beef is irradiated by electron beam and gamma-ray irradiation and its proteins assayed for amino acid content, the most sensitive amino acid to irradiation by either source is cystine. Approximately 50% of cystine is destroyed under the most damaging conditions used. Under the same conditions tryptophan shows about 10% destruction while arginine, phenylalanine, and tyrosine showed no destruction, and the rest of the amino acids show little destruction. From the cystine and tryptophan data, it appears that for electron beam irradiation the extent of destruction is not related primarily to total dose but to dose rate and particularly to energy level of irradiating dose.

While a number of reports, including reviews (*19, 20*), have appeared in recent years concerning the effect of high levels of γ-ray and x-ray irradiation on amino acids in pure solutions (*1, 2, 12, 14, 15, 16, 18, 22, 23, 25, 26, 28, 36, 42, 44, 48*), in protein solutions (*1, 6, 8, 10, 11, 13, 15, 16, 24, 29, 44, 48*) and in protein-containing foods (*3, 7, 17, 27, 31, 32, 34, 35, 39, 45, 46, 47, 49, 50, 51, 52*), few reports have appeared on the effect of electron beam and cathode ray irradiation on amino acids in pure solution (*4, 38, 41*) or in foods (*4, 37*). In no case has the effect of different energy levels of electron beam irradiation on amino acids been studied.

This paper reports the effect of both electron beam and γ-ray irradiation on the amino acids of ground beef at dose levels varying from 2–50 megarads. For electron beam irradiation, two energy levels and two power levels were used to give varying dose rates for each total dose.

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In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

Experimental

Preparation of Samples. Raw commercial beef rounds were purchased from local stores. After trimming off all of the visible fat, the meat was cut into equal pieces of approximately 2 pounds each and blanched in an electric oven, 350°F., for 1½ hours, (internal temperature, 160°F.). The pieces were then ground with a Hobart grinder using a 3/16-inch plate, canned in 3-oz. cans under vacuum, and frozen.

The cans of ground meat were divided into equal groups and irradiated according to the scheme in Table I. All samples were irradiated at ambient temperature at the Food Irradiation Facilities of the U. S. Army Natick Laboratories, Natick, Mass.

Table I. Protocol of Irradiation

Irradiation Source	*Electron Beam*				*Cobalt-60*
Energy level, m.e.v.	11		24		—
Dose rate, μamps	20	200	20	200	*10 Mrads/hr.*
Total dose, Mrad	2, 4.5, 10	2, 4.5, 10, 50	2, 4.5, 10	2, 4.5, 10, 50	2, 4.5, 10

Hydrolysis of Samples. Each sample, weighing approximately 0.03–0.05 gram and containing 12–15 mg. protein, was hydrolyzed with 500 volumes 6*N* HCl in evacuated (10 microns Hg pressure) sealed tubes at 110°C. for 22 hours. The resulting hydrolysate was dried under reduced pressure in a rotary evaporator, dissolved in 10 ml. of distilled water and dried again. The hydrolysate was then dissolved in sodium citrate buffer of pH 2.2 final concentration 15–20 μmoles nitrogen/ml. and analyzed on a Beckman Model 120B amino acid analyzer according to the method of Moore, Spackman, and Stein (*33*).

Determination of Cystine-Cysteine, Cysteic, and Tryptophan. The concentration of cystine and cysteic was too low to be measured accurately on the chromatograms resulting from the hydrolysates with final concentrations of 15–20 μmoles nitrogen/ml. By increasing the amount on the column (60–80 μmoles nitrogen), the concentration of cystine could be increased; however, the overload of nitrogen then resulted in poor resolution between cystine and valine. For this reason, the cystine plus cysteine values reported were determined both by the colorimetric assay with *p*-amino dimethylaniline (*47*) and chromatographically. The resolution of cysteic was not affected by the increased nitrogen load and was calculated from these chromatograms.

Tryptophan was determined fluorometrically by the method of Dugan and Udenfriend (*9*).

As a check on the recovery of the various amino acids, samples of bovine albumin and mixtures of pure amino acids were analyzed by the same methods as the unknowns. To check further on cystine-cysteine and tryptophan, various levels of the amino acid were hydrolyzed and analyzed in the same manner. The resulting data for albumin were compared with the values of Block and Weiss (*5*). Recovered values for amino acids obtained from chromatograms and for tryptophan by the flurometric method are listed in Table II. Values for the cystine-cysteine are listed in Table III.

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Table II. Recovery of Amino Acids by Chromatography

Amino Acid[a]	*μmoles Recovered*	*% Recovery*	*Values Listed, gm./100 gm. protein in albumin*	*Values Obtained, gm./100 gm. protein*
Aspartic	0.484 ± .005	96.8	9.1–10.8	10.4
Threonine	0.475 ± .003	95.0	5.5– 7.6	5.6
Serine	0.485 ± .000	97.0	3.8– 4.9	3.7
Glutamic	0.482 ± .007	96.4	16.1–22.4	16.1
Proline	0.509 ± .006	101.8	4.4– 5.7	4.5
Glycine	0.476 ± .007	95.2	1.7– 2.0	1.6
Alanine	0.483 ± .005	96.6	5.0– 6.8	5.4
Valine	0.482 ± .000	96.4	5.5– 6.6	5.4
Methionine	0.478 ± .002	95.6	0.8– 0.9	0.9
Isoleucine	0.489 ± .000	97.8	2.6– 3.0	2.6
Leucine	0.488 ± .000	97.6	11.4–13.9	11.4
Tyrosine	0.467 ± .000	93.4	4.4– 5.5	4.6
Phenylalanine	0.481 ± .000	96.2	6.2– 6.4	6.2
Lysine	0.477 ± .009	95.4	11.4–12.8	11.4
Histidine	0.465 ± .007	93.0	2.9– 4.1	3.3
NH_2	0.462 ± .008	92.4	—	—
Arginine	0.476 ± .005	95.2	5.5– 6.3	6.0
Tryptophan	0.495 ± .010	99.0	0.6	0.594

[a] Samples of 0.5 μmoles amino acid used in all cases.

Table III. Recovery of Cystine by Colorimetric Assay

Cysteine + Cystine (as Cystine), μmoles	*Albumin Added, grams*	*Total Cystine, μmoles*	*Amount Recovered, μmoles*	*% Recovery*
7	—	7	6.860	98.0
9	—	9	8.775	97.5
12	—	12	11.724	98.0
14	—	14	13.720	87.7
5.0	0.075 grams (20.6 μmoles cystine)	25.6	25.010	97.7
10.0	Same as above	30.6	29.990	98.0

Results

Table IV gives the analytical results obtained for all of the amino acids except cystine, cysteic acid, and tryptophan for the various irradiation treatments given in Table I as well as for the frozen nonirradiated control beef. Table V gives the assay data for cystine plus cysteine, by the colorimetric procedure, and cysteic acid. The assay data for tryptophan in Table V were determined by the fluorometric procedure.

Discussion

The data in Table IV indicate little destruction of any of these amino acids by electron beam irradiation. Following cystine and tryptophan (*see*

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Table IV. Effect of Electron Beam and γ-Ray

Grams Amino Acid/100 grams Protein ($N \times 6.25$)

Irradiation Dose					
m.e.v.	*μamps*	*Mrads*	*Aspartic*	*Threonine*	*Serine*
Control, nonirradiated			10.34	4.97	4.56
11	20	2	10.27	4.97	4.42
11	20	4.5	10.31	4.62	4.41
11	20	10.0	10.30	4.76	4.38
11	200	2	10.28	4.92	4.51
11	200	4.5	9.94	4.77	4.29
11	200	10.0	9.55	4.65	4.19
11	200	50	9.60	4.56	4.14
24	20	2	9.69	4.75	4.18
24	20	4.5	9.64	4.69	4.14
24	20	10.0	9.51	4.83	4.09
24	200	2	9.65	4.68	3.82
24	200	4.5	9.62	4.69	4.11
24	200	10	9.70	4.70	4.15
24	200	50	9.33	4.73	4.16
^{60}Co		2	10.32	5.01	4.38
^{60}Co		4.5	10.31	4.96	4.39
^{60}Co		10	10.28	4.78	4.39

Irradiation Dose					
m.e.v.	*μamps*	*Mrads*	*Isoleucine*	*Leucine*	*Tyrosine*
Control, nonirradiated			4.83	8.99	3.25
11	20	2	4.77	9.00	3.20
11	20	4.5	4.86	8.93	3.26
11	20	10.0	4.85	8.89	3.29
11	200	2	4.86	8.91	3.08
11	200	4.5	4.83	8.59	3.28
11	200	10	4.66	8.58	3.34
11	200	50	4.56	8.53	3.20
24	20	2	4.76	8.82	3.67
24	20	4.5	4.77	8.69	3.40
24	20	10	4.83	8.41	3.29
24	200	2	4.77	8.88	3.81
24	200	4.5	4.71	8.60	3.40
24	200	10.0	4.77	8.65	3.48
24	200	50	4.79	8.93	3.31
^{60}Co		2	4.96	8.99	3.38
^{60}Co		4.5	4.86	8.87	3.30
^{60}Co		10.0	4.83	8.83	3.55

Table V), the next most sensitive amino acids appear to be histidine, proline, aspartic acid, serine, glutamic acid, and valine. In these cases, the destruction amounts to about 15% in the case of histidine irradiated at 24 m.e.v., 200 μamps, 50 Mrads and 10% or less for the other amino acids. It

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Irradiation on the Amino Acids of Ground Beef

Glutamic	*Proline*	*Glycine*	*Alanine*	*Valine*	*Methionine*
16.90	4.69	4.74	6.37	5.48	2.93
16.48	4.40	4.66	6.38	5.43	2.85
16.36	4.34	4.50	6.21	5.45	2.82
16.33	4.40	4.51	6.15	5.38	2.86
16.58	4.40	4.51	6.51	5.43	2.92
16.16	4.15	4.79	6.41	5.27	2.90
15.89	4.20	4.92	5.90	4.98	2.60
15.87	4.31	4.89	6.20	5.19	2.61
15.93	4.22	4.44	6.12	5.15	2.89
15.92	4.53	4.52	6.17	5.14	2.68
15.43	4.47	4.48	6.30	5.05	2.77
15.88	4.12	4.56	6.17	4.99	2.86
15.58	4.45	4.52	6.10	4.96	2.81
15.70	4.12	4.54	6.22	5.08	2.83
15.66	4.22	4.62	6.21	5.05	2.86
16.88	4.52	4.67	6.41	5.45	2.96
16.36	4.26	4.60	6.40	5.09	2.70
16.41	4.41	4.46	6.24	5.39	2.71

Phenylalanine	*Lysine*	*Histidine*	NH_3	*Arginine*
3.85	9.53	3.43	0.71	6.73
3.67	9.44	3.43	0.75	6.70
3.70	9.45	3.45	0.69	6.70
3.52	9.45	3.43	0.79	6.79
3.69	9.47	3.44	0.80	6.71
3.55	9.39	3.32	0.74	6.76
3.58	9.45	3.30	0.79	6.79
3.61	9.39	3.20	0.84	6.80
4.27	9.26	3.33	0.79	6.74
4.05	9.28	3.32	0.78	6.79
3.83	9.06	3.33	0.79	6.51
4.35	9.27	3.20	0.87	6.84
3.91	9.20		0.85	6.77
3.94	9.26	3.03	0.85	6.63
4.00	9.21	2.94	0.94	6.85
3.98	9.49	3.44	0.75	6.70
3.65	9.44	3.41	0.94	6.71
3.93	9.42	3.46	0.94	6.74

is interesting to note that while at 11 m.e.v. 200 μamps, there appears to be progressive destruction with increasing total dose. This is much less apparent at 24 m.e.v. where frequently 2 Mrads is as destructive as 50 Mrads.

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Table V. Effect of Electron Beam and γ-Ray Irradiation on Cystine

Control, Nonirradiated	*Total Dose, Mrads*	Grams/100 grams Protein *11 m.e.v. 20 μamp*
Cystine + Cysteine		
1.27	2	1.11 (87)
	4.5	1.08 (85)
	10	1.08 (85)
	50	
Cysteic Acid		
0	2	0
	4.5	0.02
	10	0.03
	50	
Tryptophan		
1.703	2	1.665 (97.8)
	4.5	1.615 (94.8)
	10	1.595 (93.6)
	50	

The data of Table IV seem to indicate slightly less damage to amino acids with γ-irradiation than with 24 m.e.v. electrons. The marked stability of these amino acids in beef to γ-irradiation has also recently been observed by Rhodes and Southern (*39*) and is somewhat in contrast to the earlier report of Tsien and Johnson (*50*). The rather extensive losses of glutamate reported in the earlier work may have been caused by incomplete recoveries from the analytical columns or by differences in irradiation procedure. Our samples were irradiated from a cobalt-60 source, while the earlier samples were irradiated from spent fuel rods. It has recently been reported (*15*) that γ-ray irradiation of glutamic acid yields the corresponding α-keto acid, the amount obtained depending on irradiation conditions.

Our data also indicate greater stability of the amino acids in beef than was observed in the case of γ-ray irradiation of insulin. In the latter case, Drake *et al.* (*8*) found that in addition to cystine, tyrosine, phenylalanine, proline, and histidine were quite radio sensitive. Instability of tyrosine in proteins on γ-ray irradiation has also been reported by Hatano (*16*), who states that in protein, tyrosine is the most sensitive amino acid. Kolomiichenko and Morozova (*24*) found about 20% destruction of tryptophan, tyrosine, and histidine following 1.5 Mrads γ-ray irradiation of egg albumin.

Liberation of ammonia and other amines by irradiating amino acids has been reported (*7*, *15*, *17*), and a slight increase in ammonia is seen in Table IV at high levels of irradiation, whether by γ-ray or electron beam.

The data in Table V indicate more destruction of cystine than any of the other amino acids. This agrees with our earlier data on casein (*21*),

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch015

In Radiation Preservation of Foods; Josephson, E., el al.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1967.

+ Cysteine, Cysteic Acid, and Tryptophan Content of Ground Beef

Electron Beam Irradiation[a]			
11 m.e.v. 200 μamp	*24 m.e.v. 20 μamp*	*24 m.e.v. 200 μamp*	*γ-Ray Irradiation Cobalt-60*
1.04 (81)	0.92 (72)	0.82 65)	0.89 (70)
0.96 (76)	0.88 (69)	0.74 (58)	0.84 (66)
0.95 (75)	0.83 (65)	0.74 (58)	0.84 (66)
0.94 (74)		0.78 (61)	
0.14	0.13	0.23	0.11
0.15	0.12	0.077	0.17
0.194	0.07	0.11	0.20
0.214		0.15	
1.605 (94.2)	1.498 (88)	1.510 (88.7)	1.477 (86.7)
1.575 (92.5)	1.515 (89)	1.485 (87.2)	1.475 (86.6)
1.488 (87.4)	1.505 (88.4)	1.480 (87)	1.475 (86.6)
1.555 (91.3)		1.453 (85.3)	

[a] Figures in parentheses indicate percentage of control group.

in which we found complete restoration of the biological value of irradiated casein by cystine supplementation. From the data, the extent of cystine destruction appears to be related primarily to m.e.v. level, secondly to μamp level, and thirdly to total Mrad dose. An early report by Schmitz *et al.* (*43*) states that vinyl polymerization depends on dose rate, and Mead (*30*) shows that linoleic acid oxidation also depends on dose rate.

Many reports on the effect of γ-ray irradiation on free cystine and on cystine in protein and in meat have appeared, and it seems quite evident that this is one of the primary sites attacked by radiation. Gordy (*12*) has studied electron spin resonance in radiation-damaged proteins and has found two types of patterns, one for cystine arising from unpaired electrons on the protein sulfur and a different pattern for proteins with no sulfur. Markakis and Tappel (*28*) have shown that γ-ray irradiation of cystine and cysteine gives rise to H_2S, SO_4^{2-}, NH_3, and alanine (*10*). Batzer and Doty (*3*) have reported a high proportion of volatile sulfur compounds in irradiated beef, and Wick *et al.* (*52*) report 3-methylthiopropionaldehyde as the major component of these volatile substances. Others have reported methyl mercaptan (*35, 46*), methyl disulfide and isobutyl mercaptan (*34*), and ethyl mercaptan and dimethyl sulfide (*51*) as arising from protein irradiation. Sheffner and Adachi (*45*) and Niewiarowicz and Palmin (*34*) have reported decreases in cystine following irradiation of pork and beef. On the other hand, Proctor and Bhatia reported no significant destruction of cystine in fish irradiated at 5.7 Mrads (*4*) with high voltage cathode rays.

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The cysteic acid data given in Table V are less clear but seem to indicate in general the same effect as for cystine plus cysteine! The data for tryptophan show a picture similar to that for cystine, except less destruction.

If one examines in the same detail as with cystine the data in Table IV with regard to the patterns of destruction of the next most sensitive amino acids after cystine and tryptophan—i.e., histidine, aspartic acid and glutamic acid—one finds that the patterns are quite similar. They show some apparent increase in destruction between the 11-m.e.v. groups and the 24-m.e.v. groups.

Summary

Electron beam and γ-ray irradiation of ground beef have been compared with regard to amino acid destruction. The most sensitive acid to irradiation was cystine, followed by tryptophan and histidine. The greatest amounts of destruction were obtained with 24 m.e.v., 200 μamps electron beam irradiation. Electron beam density appeared to be as important as total irradiation dose in destroying amino acids. In general, little damage to amino acids and thus to the nutritive value of beef was produced by irradiation.

Acknowledgements

All irradiations were carried out by the Food Irradiation Facility of the U. S. Army Natick Laboratories, Natick, Massachusetts. Our sincere thanks are due to E. S. Josephson, Duane E. Hilmas, and many others at the Natick Laboratories for carrying out these irradiations.

This work was supported in part by contract DA-49-193 MD-2830 between the Office of the Surgeon General, Department of the Army and the University of Illinois. The opinions expressed in this publication are those of the authors and not necessarily those of the Army.

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RECEIVED November 9, 1965.

Publication Date: January 1, 1967 | doi: 10.1021/ba-1967-0065.ch015

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