ACS SYMPOSIUM SERIES **875**

Irradiation of Food and Packaging

Recent Developments

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Sponsored by the
ACS Division of Agricultural and Food Chemistry

American Chemical Society, Washington, DC

Library of Congress Cataloging-in-Publication Data

Irradiation of food and packaging : recent developments / Vanee Komolprasert, editor, Kim M. Morehouse, editor ; sponsored by the ACS Division of Agricultural and Food Chemistry.

p. cm.—(ACS symposium series ; 875)

Includes bibliographical references and index.

ISBN 0-8412-3869-3

1. Irradiated foods—Congresses. 2. Food—Packaging—Effects of radiation on—Congresses.

I. Komorprasert, Vanee. II. Morehouse, Kim Matthew. III. American Chemical Society. Division of Agricultural and Food Chemistry. IV. American Chemical Society. Meeting (224th : 2002 : Boston, Mass.) V. Series.

TP371.8.I79 2004
664'.0288—dc22 2003063662

The paper used in this publication meets the minimum requirements of American National Standard for Information Sciences—Permanence of Paper for Printed Library Materials, ANSI Z39.48–1984.

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PRINTED IN THE UNITED STATES OF AMERICA

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Preface

This book evolved from the symposium, *Food Irradiation and Packaging for Irradiated Food,* which was sponsored by the Division of Agricultural and Food Chemistry at the 224th National Meeting of the American Chemical Society, Boston, Massachusetts, August 18–22, 2002.

Food safety research has been driven by the current worldwide outbreak in microbiological food contamination. The World Health Organization (WHO) considers this microbiological contamination of food to be a serious epidemic that significantly impacts on the health of the world population. The symposium was formed to present new research on the irradiation of food and food packaging as a means to reduce and/or eliminate microbiological contamination.

The safety of food irradiation has been investigated by many national and international organizations (i.e., FDA (U.S. Food and Drug Administration), USDA (U.S. Department of Agriculture), WHO, Food and Agriculture Organization (FAO) International Atomic Energy Agency (IAEA), and Codex Alimentarius Commission) for more than 40 years. These organizations have concluded that the treatment of foods with ionizing radiation is safe and the organizations have promulgated rules and regulations for ionization's use. The food industry, however, remained disinterested in applying this technology until the FDA and the USDA, Food Safety, and Inspection Service (FSIS) approved the irradiation of uncooked red meat and meat products in 1997 and 1999, respectively. Since then, interest in food irradiation has rapidly increased resulting in investigations into the effects of this technology on the safety, quality, and shelf life of various foods and food products.

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Increased interest in Food irradiation in the United States has lead to the rapid emergence of research on irradiation of food packaging materials. Food is usually irradiated in its final package to prevent microbial recontamination. The selection criterion for these packaging materials is that they resist chemical changes when irradiated at commercial doses. A safety concern is that irradiation could lead to formation of radiolysis products in the packaging, and that these products could migrate into the food during storage. The FDA, therefore, requires that the packaging materials be evaluated and approved prior to use. The impetus behind the emerging research on packaging materials for irradiated foods is the lack of approval for modern packaging materials currently used by the food industry. FDA approval of these materials is still pending, due to the considerable lack of information on the potentially migrating low-molecular-weight radiolysis products of polymers and additives. To date, however, only a few polymers and additives have been investigated with modern analytical techniques.

The symposium provided updates on active research on irradiated foods and irradiated packaging materials. Packaging irradiation research has been insufficiently covered in the previously published books associated with ionizing radiation. The effects of the ionizing radiation on food and packaging materials are reported up to 2002 by a variety of experts from each area. The main focus of this text is on science. Other aspects of food irradiation (such as consumer acceptance, regulatory requirements, and detection methods) will be alluded to and can be found extensively covered in other books. The introductory overview chapters in this book provide background material on ionizing radiation of food and packaging materials as well as on some of the regulations involved; thus, these chapters provide the rational behind the recent and ongoing studies in both ionizing radiation of food and packaging materials. The concluding chapter is a look into the future research trends of ionizing radiation.

The audience for this book includes food scientists, packaging scientists, food technologists and engineers who are designing food packages, polymer chemists and polymer engineers who are developing new packaging materials, scientists who are interested in the radiation sterilization of medical products or in the packaging of pharmaceuticals and medical devices utilizing materials similar to those employed for

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food, as well as legislators seeking scientific information for regulatory decision making.

We acknowledge Rainer Buchalla (formerly with the Office of Food Additives Safety, Center for Food Safety and Applied Nutrition, FDA) for his invaluable inputs and assistances toward the success of the symposium and this book.

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Chapter 1

Irradiation of Food and Packaging: An Overview

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Ionizing radiation can extend shelf life and improve the quality and safety of foods. National and international organizations and regulatory agencies have concluded that irradiated food is safe and wholesome. A brief background of the food irradiation issues leading to these conclusions is given. Despite its limited use in the past, use of food irradiation is increasing as consumers are beginning to appreciate the benefits of irradiated food. Interest in the use of food irradiation increased following the 1997 US Food and Drug Administration approval of irradiation for pathogen control in unprocessed red meat and meat products. This approval led to numerous studies on a variety of food irradiation applications. Since food is usually prepackaged prior to irradiation, the possibility of radiolytic products being released from packaging materials into food requires a safety evaluation. Therefore, the use of these packaging materials is subject to regulatory review and approval prior to their use.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

I. Ionizing Radiation

Radiation for the treatment of food is achieved through the application of gamma rays (with Co-60 or Cesium-137 radioisotope), electron beams (high energy of up to 10 MeV), or X-rays (high energy of up to 5 MeV). Radiation principles explain how the gamma rays, e-beams and X-rays interact with matter. These interactions result in the formation of energetic electrons at random throughout the matter, which cause the formation of energetic molecular ions. These ions may be subject to electron capture and dissociation, as well as rapid rearrangement through ion-molecule reactions, or they may dissociate with time depending on the complexity of the molecular ion. Effects of radiation on matter depend on the type of the radiation and its energy level, as well as the composition, physical state, temperature and the atmospheric environment of the absorbing material. The chemical changes in matter can occur via primary radiolysis effects, which occur as a result of the adsorption of the energy by the absorbing matter, or via secondary effects, which occur as a result of the high reactivity of the free radicals and excited ions produced as a result of the primary effects. These highly reactive intermediates can undergo a variety of reactions leading to stable chemical products. In general, it is these chemical products that are detected and referred to as radiolysis products. For living things, these chemical changes can ultimately have biological consequences in the case where the target materials include living organisms.

II. Irradiation of Food

The use of ionizing radiation for food preservation began in the early 1920s. Later, during the 1950s-1960s, the US Army conducted research into low-dose and high-dose irradiation of military rations (*1*). These experiments prompted similar studies in other countries, and the interest in food irradiation has grown ever since. With proper application, irradiation can be an effective means of eliminating and/or reducing microbial and insect infestations along with the foodborne diseases they induce, thereby improving the safety of many foods as well as extending shelf life.

1. Safety for Consumption of Irradiated Foods

The safety of irradiated foods for human consumption has been questioned because ionizing radiation can lead to chemical changes. The wholesomeness of irradiated foods has, therefore, been the subject of considerable national and international research, which has been reviewed and evaluated by joint expert committees of the International Atomic Energy Agency (IAEA), the World Health Organization (WHO), and the Food and Agricultural Organization (FAO) of the United Nations. These expert groups have uniformly concluded that the food irradiation process does not present any enhanced toxicological, microbiological, or nutritional hazard beyond those brought about by conventional food processing techniques (*2*). These organizations, along with the Codex Alimentarius Commission and numerous regulatory agencies, have endorsed the safety of food irradiation, providing that Good Manufacturing Practices (GMPs) and Good Irradiation Practices (GIPs) are used. This has resulted in the approval of irradiated foods by many national governments, although not all of these approvals have led to use of irradiation in the marketplace.

2. Identification and Detection of Irradiated Foods

The ability to reliably differentiate between irradiated and non-irradiated foods or ingredients is in the interest of government agencies, food processors, and consumers. In addition, detection tests can be used to enforce the labeling requirements (see below) for identifying irradiated foods. Labeling will enhance consumer confidence by providing assurance of the consumer's right to choose. Furthermore, the knowledge of radiation-induced chemical changes in food provides the scientific basis for the safety evaluation of the consumption of irradiated food (*3*).

Several detection methods have been subjected to interlaboratory collaborative studies including electron spin resonance (ESR), luminescence methods, physical methods, chemical methods, and biological methods (*4, 5*). ESR measures the concentration of free radicals in irradiated matter. The luminescence methods measure the presence of excited molecules such as light emission upon heating material (thermoluminescence, TL). The physical methods are based on changes in physical properties of matter e.g. viscosity (*6*). The chemical methods are based on measurement of radiolytic products, e.g., using gas chromatography (GC) to measure volatile radiolytic products such as alkanes, alkenes and 2-alkylcyclobutanones in fat-containing food, or to measure non-volatile compounds such as 6-ketocholesterol and o-tyrosine. The biological methods are based on measurements of changes in viable microorganisms or changes in plant germination as a result of irradiation. The most practical methods are ESR (for foods containing bones, shells, or other particles), TL (for foods containing mineral dust particles), and GC (for fat-containing food) (*7*). Continuing efforts to develop detection methods are focusing on the DNA comet assay (*8, 9, 10, 11*), and the changes in protein molecular mass distribution measured by discontinuous SDS-polyacrylamide electrophoresis (SDS-PAGE) and quantified by laser scanning densitometry (*12*).

3. Labeling

Like other forms of processing, irradiation can affect the characteristics of food. Consumer choice mandates that irradiated food be adequately labeled and under the general labeling requirements, it is necessary that the food processor inform the consumer that food has been irradiated. Labeling of irradiated foods however, is undergoing reevaluation in the US. If whole foods have been irradiated, FDA requires that the label bear the radura symbol and the phrase "treated with radiation" or "treated by irradiation." Yet, if irradiated ingredients are added to foods that have not been irradiated, no special labeling is required on retail packages. Special labeling is required for foods not yet in the retail market that may undergo further processing in order to ensure that foods are not irradiated multiple times. In this regulation, FDA advises that other truthful statements, such as the reason for irradiating the food, may be included (*13*).

Because the words "radiation" and "irradiation" may have negative connotations, the labeling requirement has been viewed as an obstacle to consumer acceptance. Many in the food industry believe that an alternative wording, e.g. "electronically pasteurized," would be helpful. In 1997, Congress attempted to resolve these issues in two ways. First, it mandated that the FDA could not require print size on a label statement to be larger than that required for ingredients and second, it directed the FDA to reconsider the label requirement and to seek public comment on possible changes. The FDA had not in fact mandated a type size but did require a statement that would be "prominent and conspicuous." In response to this congressional directive, the FDA published an Advance Notice of Proposed Rulemaking (ANPR) in 1999 seeking public comment on the labeling of irradiated food, particularly on whether the current label may be misleading by implying a warning and invited suggestions of alternative labeling that would inform consumers without improperly alarming them. Thousands of comments were received, with a large number compiled into a categorical database for further examination by the CFSAN's Office of Nutritional Products, Labeling, and Dietary Supplements. This leading office for labeling policy has not yet determined whether there will be a change in labeling requirements.

4. Consumer Acceptance

Consumer advocacy groups have expressed their perception that consumers do not want irradiated food products (*14*). Consumer acceptance is based on a complex decision-making process weighing the perceived risks and benefits of food irradiation compared to the existing alternatives. The acceptance is related to the needs, beliefs and attitudes of the individual consumer and the nature of the economic, political and social environment in which food choices take place (*15*). Even though the benefits and safety of food irradiation have been scientifically documented, public awareness of such information has been limited. Consumers consequently reject food irradiation due to consumer confusion over what food irradiation is (*16*). Lack of knowledge of food irradiation and how it works generates fear that irradiated food is radioactive. Another concern is that irradiated food contains free radicals and radiolytic products. Food and health professionals could take an instrumental role in educating the consumer about the advantages and limitations of food irradiation and thus facilitate consumer acceptance of irradiated food products (*17*). The advantages of food irradiation (process safety, reduction of chemical use, and improved quality and safety of foods) over other food preservation techniques such as canning, freezing, or chemical treatment far outweigh the drawbacks - a slight reduction in nutrients (vitamins) (*18*).

Though the levels of consumer acceptance vary among countries, consumers in North America are rapidly increasing their acceptance of irradiated foods (19, *20*). Consumer education has resulted in an appreciation of the benefits of irradiated foods. Survey results indicated that consumers develop a positive attitude toward food irradiation after receiving information on product benefits; safety and wholesomeness; environmental safety issues; and endorsement by recognized health authorities. A positive response to irradiated foods can be enhanced if the consumer is allowed to compare irradiated and nonirradiated foods side by side. Increasing numbers of consumers are willing to purchase irradiated food because they prefer the advantages irradiation processing provides. Further promotion of irradiated food has been achieved by marketing tests in various countries (*21*).

5. Food Irradiation Regulations

Governmental regulation of irradiation of food varies considerably from country to country. Where irradiation is permitted, regulations are needed to license the plant, radioactive materials or process; to ensure radiation safety, environmental security, and general health and safety during plant operation; and to provide for safe disposal of any hazardous materials at the end of the operation. Each country has adopted its own unique approach to the introduction, approval, and regulation of the technology for food production. Although there is an agreement among international committee experts that food is safe and wholesome for consumption after irradiation up to a dose of 10 kGy, there is no approval for irradiation of all foods up to this limit in any country. Most countries approve food irradiation on a case-by-case basis.

In the US, the Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act (FD&C Act) of 1958 places food irradiation under the food additive regulations. It is because of this act that the FDA regulates food irradiation as a food additive and not a food process. Congress explicitly defined a source of radiation as a food additive when it stated that "Sources of radiation (including radioactive isotopes, particle accelerators, and X-ray machines) intended for use in processing food are included in the term 'food additive' as defined in this legislation." The Food Additives Amendment states that a food is adulterated (thus it cannot be marketed legally) if it has been intentionally irradiated, unless the irradiation is carried out in conformity with a regulation prescribing safe conditions of use. For clarification, the statute does not define the form of energy or the process as an additive, but rather the equipment used to irradiate the food as it may affect the characteristics of the food.

A food additive regulation, in general, may be established or amended in one of two ways: by the FDA's own initiative to propose a regulation, or in response to petitions filed by proponents of an additive's use. A petition, the more common method of regulatory alteration, is a scientific and legal document that forms the basis for the administrative record under-pinning the Agency's decision. This decision must be based on an explicit, complete, and unassailable record. The record must contain adequate information to demonstrate that the additive is safe under all conditions of use that would be permitted. When authorized, the regulation is granted generically; anyone can use the additive in conformance with the specified conditions of use permitted under the regulation.

The Food Additives Amendment does not exempt the foods that are regulated by other authorities. Meat or meat food products are subject to the Federal Meat Inspection Act. Poultry products are subject to the Poultry Products Inspection Act. Irradiated meat and poultry are then subject to the requirements of the Acts, which are administered by the Food Safety and Inspection Service (FSIS) of the Department of Agriculture (USDA). In addition, the USDA's Animal and Plant Health Inspection Service (APHIS) administers the law that quarantines certain crops from transport into the country. Irradiation is one quarantine treatment method that can be used with some foods to protect US agriculture from the import of exotic pests; therefore, such a use must also meet the requirements of APHIS.

At the recent international conference on ensuring the safety and quality of food through radiation processing (*22*), it was evident that food irradiation regulations in several countries have been or are being harmonized through compliance with the Codex General Standard for Irradiated Foods and the relevant recommendations of the International Consultative Group on Food Irradiation (ICGFI). The participants of the Conference agreed that national regulations need not stipulate maximum dose limits from a toxicological and nutritional perspective under good manufacturing and irradiation practices. The regulations should focus on the production of microbiologically safe products that meet the stated technical purposes, should provide appropriate flexibility for processors, and should be in conformity with Codex as well as the World Trade Organization (WTO) agreement on the sanitary and phytosanitary measures. These measures are required to protect human, animal and plant health and must be based on the standards and recommendations of the recognized international authorities including the Codex Alimentarius Commission.

6. Emerging Food Irradiation Applications

Irradiation is an effective form of food preservation that extends the shelf life of the food and therefore reduces the spoilage of food. The process also benefits the consumer by reducing the risk of illnesses caused by foodborne diseases. Food irradiation may be achieved using low-dose, medium-dose, or high-dose levels of radiation. Low dose irradiation (< 2 kGy) is used to delay sprouting of vegetables and aging of fruits; medium dose (between 1 and 10 kGy) is used to reduce the levels of pathogenic organisms, similar to pasteurization; and high dose (>10 kGy) is used to achieve sterility of the product. Ahmed (*23*) reported that 37 countries have approved one or more items of irradiated food products for human consumption, and 25 countries have commercialized the irradiation process.

Since worldwide foodborne diseases are increasing and attempts to reduce them have been unsuccessful, the World Health Organization considers food irradiation important toward ensuring food safety and reducing food losses (*24*). Irradiation can be a useful control measure in the production of several types of raw or minimally processed foods such as poultry, meat and meat products, fish, seafood, and fruits and vegetables (*25*). The US sets an example for the increase in permitted food irradiation uses as exemplified by the 1997 FDA approval of the irradiation of unprocessed red meat and meat products (*26*) and the 1999 FSIS/USDA approval of plant facilities (*27*). The list of FDA-approved, irradiated foods for pathogen control has recently been amended to include fresh shell eggs (*28*) and seeds for sprouting (*29*). There is continued interest in using this technology, as suggested by the pending petition submitted by the Food Irradiation Coalition to amend the permitted use of ionizing radiation to treat a variety of human foods to a maximum irradiation dose of 4.5 kGy for non-frozen and non-dry products, and 10.0 kGy for frozen or dry products (*30*).

As the outbreaks of foodborne pathogens continue, an increase of food irradiation research also continues. Irradiation is being considered as a method to ensure the hygienic quality of food, as a legitimate sanitary and phytosanitary treatment of food and agricultural commodities, as a quarantine treatment of fresh horticultural commodities, and as a substitute for fumigants in Asian countries and the USA. Low-dose and medium-dose irradiation applications are currently being investigated with food products (*31*), but the use of irradiation in combination with other processes (*32*), and high-dose food irradiations are beginning to emerge (*33*). Strategies for food irradiation continue to evolve and are updated periodically (*34, 35, 36, 37, 38, 39, 40, 41, 42*).

III. Irradiation of Food Packaging

To prevent recontamination, food is usually packaged prior to irradiation. Therefore, the effects of radiation on the food-packaging materials must also be considered when evaluating the safety of irradiated foods. Irradiation can cause changes to the packaging that might affect integrity as a barrier to microbial contamination. Irradiation might also produce radiolysis products that could migrate into food, affecting odor, taste, and possibly the safety of the food.

Many food-packaging materials are made of polymers. Radiation effects on polymers are the result of competing crosslinking or chain scission, i.e., degradation, reactions. Crosslinking is the joining of two polymer chains via a bridge-type chemical bond, leading to an increase in molecular weight. Crosslinking in many plastics and rubber is essentially a curing process that modifies the physical and mechanical properties of the polymer. Radiation-induced crosslinking dominates under vacuum or an inert atmosphere. Chain scission, on the other hand, is the fragmentation of polymer chains, which leads to a decrease in average molecular weight and dominates during irradiation in the presence of oxygen or air. Both reactions are assumed to be random and are generally proportional to dose, as well as dependent on dose rate and the oxygen content of the atmosphere in which the polymer is irradiated. Radiation does not affect all the properties of a polymer to the same degree. Therefore, when selecting a polymer for a particular application, the effect of radiation on the overall stability of the material must be considered.

1. Regulatory Requirements-Chemistry Considerations

Both crosslinking and chain scission reactions can occur during irradiation of food-packaging materials. If crosslinking dominates, the migration of packaging components is not expected to increase and, in fact, is likely to decrease compared to that observed for unirradiated packaging. In contrast, if chain scission dominates, lower molecular weight molecules are formed, and these potentially mobile molecules may migrate into food. The safety of these compounds must be evaluated because, in the U.S., all commercial facilities that irradiate food and other bulk materials such as medical supplies are currently irradiating in air. In addition, the migration of low-molecular-weight radiolysis products into food could affect the odor and taste of the irradiated food.

In the U.S., components of packaging used to hold food during irradiation must undergo premarket approval by the FDA and may be used only if they comply with the regulations in 21 *CFR* 179.45 or are the subject of an effective food contact notification or Threshold of Regulation exemption. Regardless of the review channel, chemistry data supporting the identity of and human dietary exposure to a new food-contact substance intended to be used during the irradiation of prepackaged food, as well as its radiolysis products, must be submitted to the FDA. If the packaging material is already approved for unirradiated uses, comparisons can be made to an unirradiated control to determine exposures that would result from the new irradiated use.

2. Evaluation of Irradiated Food-Packaging Materials

Studies of the effects of radiation on polymeric food-packaging materials have been limited compared to those for medical devices and pharmaceutical products. Ionizing radiation for sterilization of medical devices and pharmaceuticals provides advantages over traditional heat and chemical sterilization methods. Radiation sterilization has been successfully applied to medical products and their packaging, which is made of both thermoplastics and thermosets and includes polyesters, polystyrenes, polyethylenes, elastomers, Nylon, acrylics, and cellulose and their copolymers. Since several thermoplastics are used with both food and medical devices, similar radiation effects on these polymers are anticipated. However, the typical dose used on medical devices is 25 kGy (*43*), whereas a dose less than 10 kGy is usually applied to food. This means that the levels of radiolysis products should be proportionately lower in food-packaging polymers as compared to medical devices. The observation of radiation-induced alterations in medical products focuses mainly on the physical and performance changes of the devices. Therefore, there are limited quantitative chemical data available to aid in the analysis of the migration of radiolysis products from polymers into food. Additional investigations are needed to evaluate the suitability of modern food-packaging materials and adjuvants intended for use during the irradiation of prepackaged food.

Most of the packaging materials listed in 21 *CFR* 179.45 are films and homogeneous structures that were approved in the 1960s. These materials do not fully meet today's needs, as modern materials are more desirable to the food industry. Many modern materials have not yet been evaluated by FDA. These materials may contain adjuvants that prevent undesirable reactions from occurring during polymer processing and subsequent irradiation. Adjuvants may be added to minimize the loss of chemical and physical properties, e.g., antioxidants are added to polymers to prevent the polymer from oxidizing, UV stabilizers are added to prevent discoloration of polymers when exposed to light, and release agents are added to enable high-speed production. Adjuvants are especially prone to degradation upon irradiation because they degrade preferentially over the polymer. Therefore, the radiation-induced degradation of various polymer adjuvants, including antioxidants, plasticizers, coatings, release agents, and stabilizers must be evaluated as well (*44, 45*).

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Chapter 2

Food Irradiation and Marketing in Thailand

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Since 1964, Office of the Atomic Energy for Peace (OAEP) has been responsible for doing research and development on gamma irradiation of foods to reduce spoilage, disinfest and extend shelf life, leading to significant cost savings to the food industry. In early 1984, OAEP proposed an economic feasibility study for setting up a commercial scale food irradiation plant. Market testing of irradiated frozen shrimp, frozen chicken, onion, fermented pork sausage (Nham), made from raw pork, and mungbeans were then conducted. The Thai Irradiation center, a multipurpose food irradiation plant, was commissioned in 1989 for food irradiation technology transfer. It was planned to provide irradiation services to eight food items including onion, potato, garlic, salted and dried fish, mungbeans, fermented pork and sausage at a rate of 0.3-6000 tons per year. Irradiation services are available to other commodities such as spices, herbs, enzymes, dried fruits, medical and pharmaceutical products. Past, present, and future commercialization of irradiated food in both domestic and international trade is discussed.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Introduction

Losses of agricultural products in Thailand are due to hot climate that accelerates the ripening of fruits and sprouting of vegetable, and the growth of spoilage microorganisms, pathogenic microorganisms, and insect infestation.

Onion, garlic, and potato have a short shelf life, and they cannot be stored long enough for off-season domestic consumption. The annual production and domestic consumption in 1983 of onion in Thailand was approximately 50,000 and 30,000 tons, respectively. However, about 50% of the harvest was discarded during storage because of rotting and sprouting. Fresh onion can be stored for only a few months at ambient tropical conditions. During the scarce season of 1982, Thailand had to import 4,760 tons of onion at a cost of 56 million bahts. The currency in 1982 was 26 bahts for a US dollar.

Garlic is one of economically important agricultural commodities in Thailand. Although it can be stored in the dry stage, it can last only 3-5 months. Garlic of about 37.7 million bahts value was imported during the off-season (July-December) in 1977. The price of garlic per kilogram (kg) at the farm was 14.1 bahts compared to the wholesale price of 41.4 bahts during off-season.

Mungbean is an economically important cereal in Thailand. The production in 1983 was 20,000 tons. The untreated produce sometimes is totally infested by insects within 3 months.

Insect damage reduces the market value of dried fish products, and also results in loss of nutritional value. Adjusting market prices can be made by mark-ups to compensate with the product losses. Besides lower product quality, the higher prices will burden consumers.

Fresh food infected with parasitic materials is a problem worldwide. Several diseases of parasitic origin have high morbidity. Opisthorchiasis, gnathostomiasis and cysticercosis are good examples of food borne diseases caused by eating raw fish and pork, which lead to high morbidity. Angiostrongyliasis and trichinosis can be fatal to those who have eaten raw Pila snails or wild boar. Attempts have been made to prevent these parasitic diseases by properly cooking the food. Nevertheless, the consumers who prefer the taste of raw meat may not accept control measures by boiling, grilling and frying.

One major problem for fruits is short shelf life and insect infestation. Due to insect infestation and microbial contamination, importing countries will reject some importing food items. This becomes a considerably economic loss. In recent years, there is an increased interest in the potential of exports of fresh fruits from Thailand. Therefore, the level of production of several tropical fruits is rising, and the economic benefits that would result from such increased exports are obvious. However, the previously localized occurrence of various insect pests had led to the establishment of quarantine barriers against the free movement of fresh fruit and other agricultural commodities. Typically, the export of fruits from Thailand to foreign markets in Japan, Australia and the USA is being restricted by the occurrence of the Oriental fruit fly (*Bactocerlus dorsalis*) and the melon fly (*Dacus curcubitae*). These quarantine restrictions for the movement of fruit can be overcome technically by radiation disinfestation treatment. Conceptually, such the treatment may be applicable to other insect pests as well.

Research and Development

The Office of Atomic Energy for Peace (OAEP) was established in 1962. By mid-1963, an installed research reactor reached its maximum power of one megawatt for the first time. During the second half of 1963 and in 1964, the reactor was not fully operated at its full power. Early experiments in connection with food irradiation were not carried out until near the end of 1964. Preliminary irradiation experiments for disinfections were performed on lime, rice bran and rice grain. This reactor was the only a gamma source for food irradiation until early 1966.

The Food Science and Entomology sections, Biological Science Division, at OAEP were strengthened in 1966 through the technical assistance of IAEA experts, Drs. D.N. Rhodes (UK) and R.L. Beard (USA), and after acquisition of a new irradiation unit of Co-60, 8 kCi (0.29 PBq). The gamma cell arrived in Bangkok in March 1966 and it has been the sole gamma source used in food irradiation and related entomology studies until 1971. Because the irradiation chamber was small with an internal volume of 2 liters, a maximum of 6 bananas could be loaded at a time. The study on papayas had also been limited to only small-size varieties.

Later, the IAEA provided technical assistance in the fields of fruits and fish. At the start of the study on seafoods, cooperation on certain aspects of microbiology was obtained from the Applied Scientific Research Cooperation of Thailand and the Faculty of Veterinary Science at Chulalongkorn University. As the work on microbiology increased, a small laboratory was also set up at the OAEP in 1970.

In 1969, OAEP acquired a 30 kCi, cobalt-60 irradiation unit (Gamma-beam 650 Type IR31) from the Atomic Energy of Canada Ltd. (AECL). The International Atomic Energy Agency (IAEA) kindly contributed half of the cost of the irradiation unit under the Agency's programme of technical assistance. The installation was completed in November 1971. The new irradiation source was used for demonstration to trainees of the IAEA Inter-regional Training Course on Dosimetry for Industrial and Agricultural Radiation Processing Establishments between November 15 and December 10, 1971. This new facility offered opportunities to study irradiation of materials in larger quantities.

In 1978 the gamma-beam 650 was overhauled and reloaded with a 50kCi (1.85 PBq) Co-60 source, and put in operation again in 1981. Since then research on both basic and applied aspects of radiation preservation of various food items of commercial importance have been carrying out actively.

Numerous studies on food irradiation have been undertaken at the OAEP in the past four decades. These included irradiation to decontaminate spoilage and pathogenic microorganisms in fresh and frozen seafood, poultry, meat and meat products, spices, herb, and enzymes, as well as to extend shelf life of certain fresh seafoods, delay ripening of some local fruits, and control of insect pests in stored grains and fruits. The results of some of these investigations were summarized and are shown in Table I. Food irradiation offers wide applications for treating many types of food for different purposes (Table II).

During 1975-1979, studies on the wholesomeness test of raw fermented pork sausage (Nham) were conducted as a joint, collaborative project with organizations under the Ministry of Public Health, Ministry of Industries, Kasetsart University, Mahidol University, and Chulalongkorn University. This project was supported by the Subcommittee of Wholesomeness Test of food Irradiation, which was under the Thai Atomic Energy Commission, to promote the safe consumption of this fermented pork sausages produced by home industry. Based on toxicological studies, the mice fed with fermented pork sausage showed no adverse effect. The results of this study strongly encouraged the OAEP scientists and a market trial for this sausage to be conducted in 1986.

Feasibility Studies

In early 1984, OAEP initiated an economic feasibility study for setting up a commercial scale food irradiation plant. In this study, a multipurpose agricultural pilot-plant demonstration facility was operated by the staff of OAEP. However, commercial success for any potential food irradiation application requires positive response to the questions concerning technical, economic, and regulatory issues, need fulfillment, marketability, volume logistics and feasibility in comparison with other alterative technologies. These questions are criteria for

Table I. Research on Food Irradiation Conducted at the Office of Atomic Energy for Peace (OAEP)

Products	*Purpose*	*Temp. °C*	*Dose, kGy*	*Storage time*
Banana (Musa paradisiaca L.)	Shelf-life extension	17	0.2-0.4	7 days
Mango (Margifera indiac Linn.)	Shelf-life extension	17	0.3-0.5 Hot water dip	28 days
	Fruit flies Quarantine[1]	18	0.3	15 days
Tangerine	Fruit flies Quarantine[1]	18	0.15	28 days
Lychi	Quarantine[1]	10	0.2	9 days
Sweet Tamarind	Insect disinfestation[2]	Ambient	1	6 months
Onion (Allium cepa L.)	Sprout inhibition	10	0.09-0.1	6 months
Potato (Solanum tuberosum Linn.)	Sprout inhibition	10	0.12-0.15	6 months
Ginger (Zingiber officinale Rose.)	Sprout inhibition	20	0.04-0.06	6 months
Mushroom (Voloaria esculenta Bresadola)	Growth inhibition	17	0.5-1.0	4 days
Rice (Oriza sativa)	Insect disinfestations[2]	Ambient	0.3	> 1 year
Mungbean (Vigna radiata (L.) Wilezed)	Insect disinfestation[2]	Ambient	0.3	> 1 year
Chub Mackerel	Shelf-life extension	5	2.0	3 weeks
Boiled Chub Mackerel	Shelf-life extension	25-30	2.0-3.0	2 weeks
Salted and Dried Fish	Insect disinfestations[2]	Ambient	0.3	> 6 months
Frozen Shrimp	Decontamination[3]	-18	3-4	6 months
Fish Meal	Decontamination[3]	Ambient	5.0	6 months
Crab Meat	Shelf-life extension	3	1.5-2.5	3 weeks
Fermented Pork Sausage	Decontamination[3]	Ambient	2.0	3 weeks
Vienna Sausage	Decontamination[3] Shelf-life extension	4-6	2.8	3 weeks
Vietnamese sausage (Mu Yaw)	Shelf-life extension	4-6	1.8	113 days
Chicken	Decontamination[3]	18	3	> 6 months
Spice	Decontamination[3]	Ambient	10-20	> 6 months
Fish[4]	Liver fluke disinfection		0.3	

[1] Quarantine purpose is to facilitate exporting fresh fruits destined foreign markets where restriction on pest quarantine apply.

[2] Disinfestation purpose is to reduce losses due to insect damage in food and agricultural products.

[3] Decontamination purpose is to decontaminate pathogenic microorganisms.

[4] Cooperative project between OAEP and Faculty of Tropical Medicine, Mahidol University.

Table II. Applications of Food Irradiation. Data Obtained from OAEP Research

Effect of Treatment	*Dose (kGy)*	*Example of Food*
Low Dose (up to 1 kGy)		
(a) Inhibition of sprouting	0.05-0.15	Potatoes, onions, garlic, Ginger-root, etc.
(b) Insect disinfestation and parasite disinfection	0.15-0.50	Rice, mungbean, fresh Fruits, raw and dried fish
(c) Delay of physiological process	0.50-1.0	Fresh fruits and vegetables
Medium Dose (1-10 kGy)		
Decontamination of spoilage and pathogenic microorganisms	2.0-5.0	Fresh and frozen seafood, poultry and meat in raw or frozen state, etc.
High Dose (10-50 kGy)		
Decontamination of spoilage and pathogenic microorganisms	10-20	Spices

identifying the specific and the potential for food irradiation commercialization in Thailand. Food irradiation service was planned to provide the public the irradiation of eight selected food items at the annual rates as follows. The annual rate of irradiation of onion, potato, and garlic was approximately 6000, 2000, and 6000 tons, respectively; 1000 tons for salted dried fish and smoked fish, 3000 tons for mungbean, and 0.3 tons for fermented pork sausage and Vietnam sausage. Revenue was approximately 15.6 million bahts compared to the fixed cost of 45.5 million bahts and annual operating cost and expenses of 6.67 million bahts. Irradiation services would be available to other commodities as well. Based on these value estimates, the project would be economically viable and would obtain an adequate return from the capital investment (where the interest rate of return was 16.59%). In addition, the benefit-cost ratio was 1.12 at an interest of 12%. Details of the variable costs and incomes for each of the irradiated commodities are as follows.

Onions

Sprout inhibition of onions by gamma irradiation project was conducted at OAEP. Onions irradiated at 30-120 Gy showed negligible percentage of sprouting after storage at 12°C for 6 months. There was no significant weight loss of irradiated onions in comparison with non-irradiated onions stored under the same conditions. Upon the recommendation of the Thai AEC, the Ministry of Public Health has approved irradiation of onions in 1973. Upon request by businessmen in 1974, the gamma-beam 650 radiator was extensively used to irradiate onions of approximately 700 tons. The irradiation cost was 0.25 bahts/kg (20 bahts = US$1 in 1973). The request for service has increased as these businessmen realized the profit from onion irradiation, which has proven to be economically feasible. The total cost for irradiating freshly harvested and cured onions from farms, including transportation, labor, packaging, cold storage and interest, is approximately 7,228 bahts/ton. After storage for 6 months, it is estimated that the income from selling 60% of remaining irradiated onion is 9,000 bahts. Therefore, the profit is 1,772 bahts/ton or approximately 1.77 bahts/kg. This calculation is based on the information obtained from businessmen. If the price of onions from farms is 1 baht/kg instead of 2 bahts/kg and the market price is 20 bahts/kg instead of 15 bahts/kg, the profit would be 7.77 bahts/kg instead of 1.77 bahts/kg. Apparently, irradiating onions is economically feasible.

Garlic

Research conducted at OAEP showed that the shelf life of garlic can be extended for more than 6 months when irradiated at 100 Gy and stored at 10°C. The variable costs of irradiation for sprout inhibition of freshly harvested and cured garlic from farms include transportation, labor, packaging, irradiation service charge, cool storage at 10°C for 6 months, and the loan interest, which have been estimated to be 20.626 bahts/kg. After storage for 6 months, it is estimated that the income from selling the marketable garlic (70% of the totally irradiated) is 28,939 bahts. The profit is 8,333 bahts/ton or approximately 8.3 bahts/kg. This suggests that a packer/distributor will be able to make a reasonable profit. Irradiation of garlic is economically feasible.

Mungbean

Radiation is an alternative to insecticide for controlling insect infestation. Research at OAEP has indicated that irradiation at 0.3 kGy can disinfest insect pest in mungbean. The prices of mungbean at the farms and the wholesale prices after storage for 2 months are approximately 5,500 and 8,500 bahts/ton, respectively. The cost of irradiated mungbean after storage for 2 months is 7,277 bahts/ton. Therefore, there is a profit of 1.22 bahts/kg. Irradiation of mungbean is also economically viable.

Salted Dried Fish and Smoked Fish

Salted dried fish and smoked fish are a good protein source for the people of Thailand, but the products are usually infested by insects that lay eggs on these products while drying. Insects damage about 10-30% of these products. Research conducted at OAEP has indicated that both salted, dried fish and smoked fish irradiated at 0.3 kGy are free of these insects. The estimated cost for irradiating fishery products is 1.75 bahts/kg, of which 1.50 and 0.25 bahts are for irradiation service and packaging, respectively. However, the additional cost is still lower than an approximate of 8 bahts/kg of loss due to insect damage, which is calculated based on 20% damage of the fishery product price at 40 bahts/kg. Therefore, irradiation of the fishery product is economically feasible because of the low irradiation cost and the reduction in product loss.

Meat Products

Nham (fermented pork sausage) and other sausages are often contaminated with *Salmonella*. Research conducted at OAEP indicated that radiation dose at 2 kGy can decontaminate *Salmonella*. Therefore, the irradiation service of the multipurpose food irradiator can also be used to radicidize *Salmonella* as well as to extend the shelf life of Mu yaw (Vietnamese sausage) for over 20 days during storage at 2°C.

Thai Irradiation Center

With the contribution of the Canadian International Development Agency (CIDA), a multipurpose food irradiator named Thai irradiation center (TIC) was established in 1989. This radiator was designed at full capacity of 3 MCi but initially loaded with only 400 kCi (14.80 PBq) Co-60. This center provides opportunities to fulfill the objectives of the governmental research and development including market trials on certain irradiated food and agricultural products and the transfer of the technology of irradiation processes to commercial sector. The Thai irradiator is a CARRIER TYPE GAMMA IRRADIATOR MODEL JS-8900, designed by Nordion International Incorporation. An automatic conveyor system transfers the product by a pneumatically driven mechanism through the maze into the irradiation room and around the Co-60 source. The plant can be operated in three modes: continuous, batch, and incremental. The batch consists of 9 carriers through the irradiation room without interrupting the operation of the irradiator. The Computerized Irradiation Monitoring System (CIMS), microprocessor system, generates documentation on timing and monitoring of the machine operation.

Market Tests of Irradiated Foods

Market tests were conducted for six irradiated foods including frozen shrimp and chicken, onions, garlic, rice, and sweet tamarind. The results showed a very positive response from consumers. Since 1984, these irradiated food items (with irradiation labels) have been sold in shops, supermarkets and various government offices in Bangkok for market trials to determine consumer acceptance. At present, the irradiated Nham for decontaminating pathogenic microorganisms and irradiated tamarind for insect disinfestation are available in several markets nationwide.

With the recommendation of the Thai Atomic Energy Commission, the Ministry of Public Health issued a ministerial regulation No. 26 on Food Under Control declaring that irradiated food was subject to control starting from 10 March 1971. This regulation helped consumers to have choices of buying irradiated products if they felt it was safe for consumption. Irradiated onion was the first food item, which was approved by Ministry of Public Health for market testing in collaboration with an onion trader in Thailand. In 1979, Thai FDA promulgated Notification No.9 and Notification No.10 to prescribe irradiated food and irradiated onion, respectively, as the specially controlled food. The most recent clearance of irradiated food is the Notification No.103, which was approved by Thai FDA in 1986. Under this Notification, 18 irradiated food items have been approved for human consumption.

Irradiated Frozen Shrimp and Chicken

552 kilograms of irradiated frozen shrimp and 933 kilograms of irradiated frozen chicken with irradiation labels were entirely sold out in five of the country's leading supermarkets in Bangkok during 1984 - 1985. The products were irradiated at a minimum dose of 3 kGy for eliminating pathogenic microorganisms. A poster was displayed at the selling area to inform consumers about the benefits of irradiated food and its wholesomeness. Questionnaires were also given to buyers to survey the consumer attitude toward irradiated frozen shrimps. The survey results showed that 98% of the buyers were satisfied with the quality of irradiated frozen shrimps. The comments were both positive and negative. The positive comments were: the irradiated products were fresh, clean, pathogen-free, and longer storage life. They would support these market trials and buy other irradiated food items as well. 69% of the surveyed buyers indicated that they had some information about irradiated food before and they would buy this product even with the irradiation label. The negative comment was mainly the higher price of the irradiated product compared to the non-irradiated one.

Irradiated Onion

Pre-commercial scale irradiation of onion for marketing trials was conducted again in 1986 after the first trial in 1973. Approximately nine tons of onion were irradiated at an average dose of 90 Gy and subsequently placed on the market for trials conducted by the OAEP, in collaboration with an onion trader during September-November 1986. The onion was shipped to five locations (an open market, three shops, and one department store in Bangkok) at the regular intervals with the quantity depending on the rate of sale at each location. A number of questionnaires were also given to the shops and department store for distribution to buyers.

The market test results showed no negative comment. The majority of the customers indicated that they would buy irradiated onion again because of its superior quality to the non-irradiated one. About 99% of irradiated onions were sold out at retail prices. Due to the success in storage and marketing of irradiated onions in the previous year, two onion traders requested the OAEP to irradiate 330 tons of onions in 1987. Moreover, the tonnage of irradiated onion had increased to approximately 500 in 1988 with the involvement of three onion traders.

Irradiated Fermented Pork Sausage (Nham)

Nham is a fermented pork sausage, made from raw pork, which is normally consumed without cooking or any heat treatment. This product is often contaminated with *Salmonella* and occasionally with *Trichinella spiralis*. The research conducted at the OAEP demonstrated that irradiation with 2 kGy dose could eliminate the risk from these pathogens.

Market trial of irradiated Nham has been conducted by the OAEP since 1986. Irradiated Nham was packaged with label showing food irradiation logo Radura and the purpose of irradiation: to eliminate parasites and pathogenic microorganisms. Irradiated and non-irradiated Nham packages were displayed side-by-side at the Mah-Boonkrong supermarket in Bangkok for eleven weeks. With the same product weight, irradiated Nham was sold at 13 bahts/package versus 12 bahts (25 bahts/ US dollar in 1986) for non-irradiated one. The results obtained from this market trial indicated that irradiated Nham were sold ten times more rapidly than non-irradiated product even at a higher price of one baht per package.

Consumer attitudes toward irradiated Nham were evaluated based on 138 questionnaires received from the buyers. About 78% of them could not differentiate the texture and taste of the irradiated Nham from the non-irradiated one. 92% of them would buy irradiated Nham again. Even the price increased by 1 and 2 bahts, 79% and 71% of the customers respectively, still wanted to purchase the irradiated Nham. Market trials of irradiated Nham continued and the number of shops and stores participating increased to more than 12 supermarkets and many more government offices in Bangkok in 1991.

Sweet Tamarind

The effect of gamma irradiation at 1-6 kGy doses on quality improvement of sweet tamarind was studied. The results suggested that 1 kGy dose was sufficient for eliminating all insects that infested in sweet tamarind. Market trials of irradiated sweet tamarind were carried out during 1995-1996. One kilogram of irradiated sweet tamarind was packaged with a questionnaire inserted inside the package, and it was sold in Bangkok and some provinces in the Northeastern part of Thailand. All 1,400 packages of irradiated sweet tamarind were entirely sold out. However, only 47 completed questionnaires were received and evaluated. The results showed that 98% of the surveyed consumers were satisfied with the quality of irradiated sweet tamarind in terms of insect free and 75% of them indicated that they would buy the irradiated tamarind again.

International Market Development

International market development for irradiated food and nonfood products was also tested. Shipping trials of several foods and agricultural commodities were conducted. Irradiated frozen shrimps were shipped in a commercial simulation practice to Netherlands in 1983, to Australia in 1985, and to Canada in 1992. The results showed no problem of pathogenic microorganisms in the shipped frozen shrimps. Irradiated mangos were also shipped to Canada in 1992. The shipping trials of both irradiated mangos and frozen shrimps were parts of the petitioning protocol for marketing the two commodities in Canada. In addition, shipping and marketing trial of irradiated Thai orchids to Australia was also conducted in 2002 and the results are being evaluated.

Ministerial Regulation

In response to the recommendation of the Thai Atomic Energy commission, the Ministry of Public Health (MPH) issued a ministerial regulation No. 26 on 'Food Under Control' declaring that irradiated food is subject to control starting on 10 March 1971. On the basis of the recommendation and in the interest of improving the regulation on food irradiation to be most suitable for the social and economic situation, the MPH has promulgated Notification No. 103 (in 1986) on the Prescription of Manufacturing Process for Irradiated Food, which will permit food to be irradiated at an effective dose but not more than overall average dose of 10 kGy on some food items. This notification follows Codex General Standard for Irradiated Foods and Recommended International Code of Practice for the Operation of Radiation Facilities used for the treatment of foods as guidelines. It also prescribes the control process for good irradiation practice (GIP), which includes food standards, packaging, and labeling. Radiation facilities must be operated in full compliance with the licensed premise, by the licensed premise, and must conform to good irradiation practices such as those issued by International Consultative Group on Food Irradiation (ICGFI). Irradiated food must be labeled with registration numbers, the International irradiation symbol (Radura), purpose of irradiation, name and address of manufacturer and operators of the radiation facilities, and date of irradiation.

All manufacturers and importers are required to have manufacturing licenses and importation licenses from the Thai FDA. At present, registered food items are pork sausage, sweet tamarind, onions, spices and seasoning.

Association of South East Asia Nations (ASEAN): a Harmonized Regulation

With regard to the acceptance and implementation of a harmonized regulation as described in the final Draft for a Harmonized Regulation on Food Irradiation for ASEAN, the Thai Food and Drug Administration (FDA) and the Office of Atomic Energy for Peace (OAEP) generally agree with most of the draft's content. However, there are some reservations on two issues: (1) The minimum effective dose should be specified in each class of food in order to adequately control pathogenic microorganisms and parasites, and (2) The percentage of irradiated ingredient present in the food in which labeling would be required should be clearly specified. It is anticipated that the acceptance and implementation of this harmonized regulation will facilitate international trade of irradiated food in ASEAN. This regulation may be accepted by countries in other regions as well.

Export of Agricultural Products

Exports of agricultural products including fresh fruits and vegetables are one major source of foreign exchange for Thailand. However, only a few tropical fruits can gain access to lucrative markets in Australia, Japan and the USA because of infestation of quarantine pests, especially fruit flies. Since irradiation is recognized as an effective, board spectrum quarantine treatment against various species of fruit flies and other insect pest regardless of host commodities, Thailand needs food irradiation technology for the opportunity in developing foreign markets for her products. It is anticipated that implementation of national regulations on food irradiation and application as a sanitary and phytosanitary treatment of food and agricultural commodities will increase for both domestic and export markets, subsequently promoting an international trade of irradiated foods and agricultural produce.

At present, the private sector is very interested in setting an electron-beam and X-ray plant for irradiation of fruits in Thailand, similar to the Hawaiian facility that is located in Hilo on the Big Island. This plant will serve as a unique postharvest treatment facility for fresh fruits destined for export to foreign markets where restrictions on pest quarantine apply.

Conclusion

Food irradiation in Thailand has been proven technically and economically viable for various foods and food products. Commercialization of several irradiated foods has been successful, such as irradiated fermented pork sausage and sweet tamarind, which are very much in progress. Revenue from irradiation service for exporting food and other commodities is approximately 5.55 million bahts (42 bahts = 1US$ for a recent exchange rate). Irradiation can be applied to other food products and commodities of importance to the economy of Thailand, offering a possible increase in international trade of these products, and helping sustain the incomes of farmers/growers, exporter, and food irradiation plant owners. Irradiation is considered the best possible, post-harvest treatment for assuring the quality and freshness of fruits and vegetables. As an effective phytosanitary treatment, irradiation of fruits and vegetables is expected to increase in countries where insect pests and microbial contamination are a major problem.

Chapter 3

Electron Beam Fluidized Bed Processing of Foodstuffs

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The application of EBFB process for insect pest management in stored products offers the advantages of a physical, in-line process, potentially effective at all life stages (unlike fumigants that perform poorly on eggs). The challenge is the ability of the energetic electrons to reach life stages of the insect (largely larval), which may be living deep inside the endosperm of the seed. This work has concentrated on the rice weevil (*Sitophilus oryzae*) and the lesser grain borer (*Rhyzopertha dominica*). The larvae of both species are "internal feeders" in grain, notably in wheat, corn and rice. Results are presented for efficacy of the EBFB process as a function of energy and dose for all life stages of these insects. Examples are presented of the application of the insect population growth models developed at the USDA-ARS (Grain Marketing and Production Research Center) which were used to evaluate the effectiveness of the process when used on stored products (winter wheat) containing mixed life stages of *R. dominica*.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Introduction

The electron beam fluidized bed technique (*1*, *2*) has been developed in this laboratory for the physical treatment of stored products. Its major advantage lies in its isotropic illumination of a particle (e.g. a grain kernel) during transport through the treatment zone. Pneumatic conveyance techniques are well developed for the handling of feed grains and seeds (*3*), so that their high-velocity transport, say 1000 m/min, is practicable. As a consequence, high throughputs are possible with modest (air) loading factors in the process stream. The result is that lower electron energies can be utilized with significant improvements in process economics.

Pest control should ideally eliminate the population. After pesticide application, some survival is likely in practice, due either to improper application or genetic resistance (*4*). This results in the development of resistant populations, often in only a few generations. These chemicals are typically stomach, nerve or respiratory poisons; whereas, ionizing radiation is non-site selective and depends little on the metabolic state of the insect. The general approach for the application of radiation disinfestation has been to utilize a dose adequate for sterilization (usually resulting in death) of the insect at all life stages. An important part of our study was evaluating the electron penetration requirements for reaching the early life stages of these internal feeders.

This study focused on the most prevalent and damaging insect pests of whole grains and seeds. Most stored product pests feed on available starch of broken or ground-up seeds and grains. Few species can penetrate the seed coat (pericarp) or deposit eggs (oviposit) inside intact kernels. Those that can are: rice and granary weevil, the lesser grain borer, the Angoumois grain moth and several species of seed beetles. Table I lists the most prevalent domestic (U.S. and Canada) pests of these internal feeding species, (along with the life cycle consumption of each species). This paper focuses on perhaps the most damaging grain pest in storage and transport, the lesser grain borer. Like its relatives, the *Bostrichids*, most of which are wood borers, it has strong jaws and completes the immature stages of its life cycle inside the endosperm of the grain kernel (*5*).

Much of this study has been devoted to assessing the two important parameters of the EBFB process, namely energy and dose. The process must be efficacious for all life stages and clearly the most difficult are those larval stages during which the insect is living and feeding deep inside the grain kernel. Relatively low energy electrons easily access the eggs (deposited near or on the seed pericarp) as well as the adult stages; it is the larvae and pre-emergent pupae, which present the penetration challenge. For wheat

Table I. Relative Consumption for Stored Product Insects

Species	*Common Name*	*Diet*	*Total Lifetime Consumption (mg)*
Tribolium castaneum	Red Flour Beetle	Flour	328
Prostephanus truncatus	Larger Grain Borer	Corn	236
Rhyzopertha dominica	Lesser Grain Borer	Wheat	154
Sitophilus granarius	Granary Weevil	Wheat	86
Sitophilus Oryzae	Rice Weevil	Wheat	32

SOURCE: Reproduced from reference 5. Copyright 1988.

and rice, the protective pericarp for these life stages may be in the 400-800 µm range, or, for hard winter wheat (ρ=1.4 g/cm^3), a thickness of 560-1100 g/m^2. Typical electron penetration depths for electron processors working at moderate voltages are presented in Figure 1.

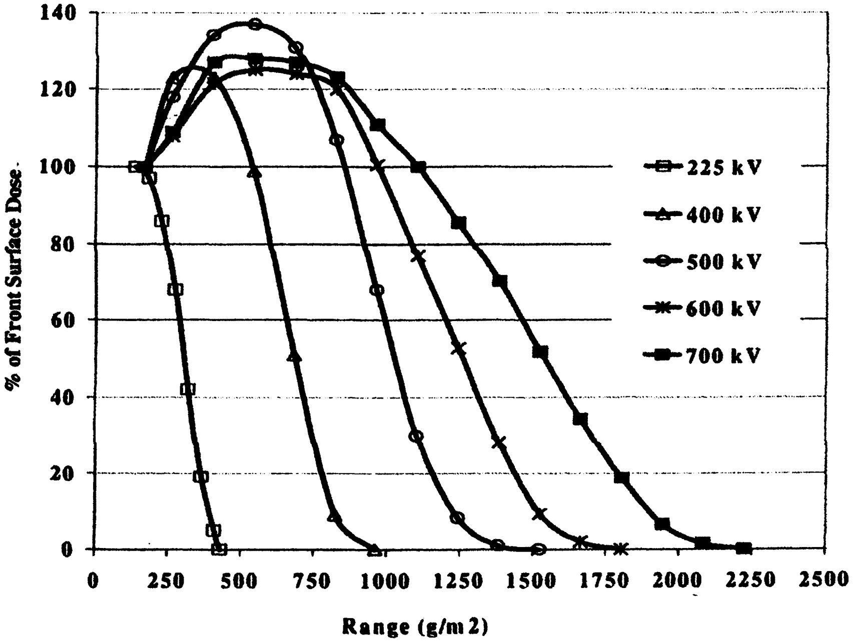

Figure 1. Penetration Curves as a Function of Voltage

Methods and Materials

Two systems have been used for this work over the past four years of this study, namely the self-shielded 240 kV pilot system located at EPS (N. Billerica, MA), and an adaptation to a higher energy vault shielded (500 kV) scanned system located nearby. This 500 kV operating level can also be self-shielded to accommodate the installation requirements of the food and feed processing industries. A schematic of the first system is shown in Figure 2 while the feeder-treatment channel used on the 500 kV accelerator is shown in Figure 3. A vibratory feeder has been used to control the feed rates used in these trials, while product recovery utilized a bag-filter receiver evacuated by a 5 horsepower regenerative blower (*3*).

In verifying the effectiveness of this process for all life stages, attention was paid to ensuring that the "weaknesses" of fumigation were overcome. Table II illustrates some of the limitations of fumigation with Phosphine, namely very low mortality at reduced temperatures, particularly with the egg and adult stages (*6*). The average duration of these various life stages for *R. dominica* (lesser grain borer) is shown in Figure 4 for the 45-day life cycle for our work at 30°C. As expected, the early life stages show high resistance to fumigants and the early larval stages (instars) are better protected from external electron treatment due to this "protective shielding" effect. Figure 5 shows data for various larval stages of *S. oryzae* (rice weevil) as a function of electron energy at

October 23, 2009 | http://pubs.acs.org
Publication Date: January 2, 2004 | doi: 10.1021/bk-2004-0875.ch003

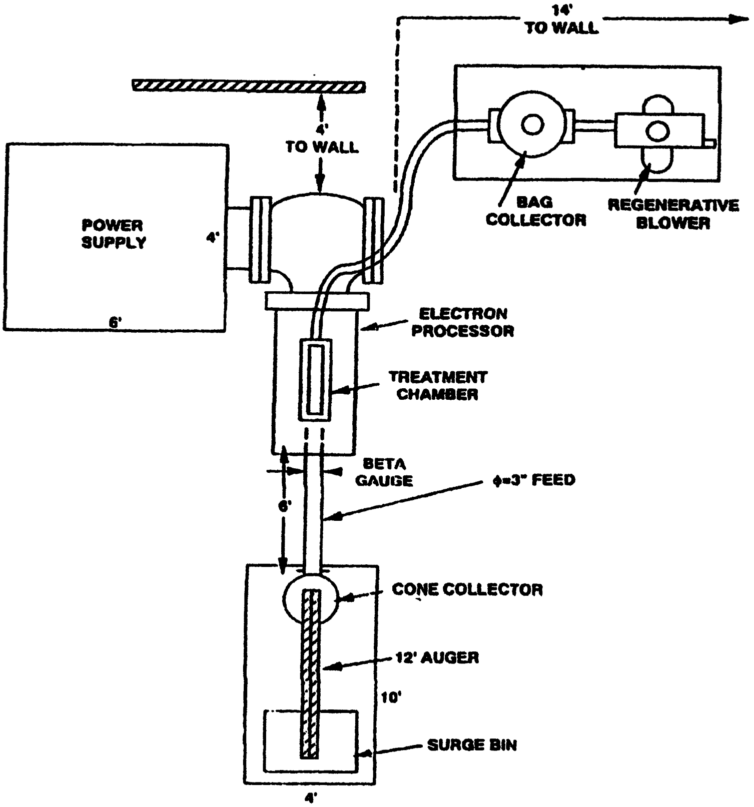
14' TO WALL
4' TO WALL
POWER SUPPLY
4'
6'
BAG COLLECTOR
REGENERATIVE BLOWER
ELECTRON PROCESSOR
TREATMENT CHAMBER
BETA GAUGE
ϕ=3" FEED
6'
CONE COLLECTOR
12' AUGER
10'
SURGE BIN
4'

Figure 2. Schematic of the EPS ebfb Pilot System with Auger Feed

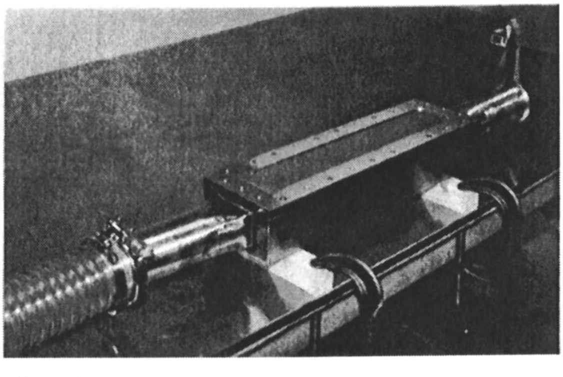

Figure 3. The 7.6 cm x 45.7 cm Treatment Duct for EBFB Studies to 550 kV

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

fixed dose (*7*). This insect has a life cycle similar to that of *R. dominica*, and the reduced protection of 7 to 12 days extra time inside the kernel is obvious. For example, mortality at 230 kV is doubled with irradiation at 22 days rather than at 14 days. The times indicated are the periods after oviposit. The ratio of mortality over this same period at 400 kV is 6 (30 vs. 5), again showing the higher mortality as the larvae proceed through their molt stages and reduce the surrounding protective kernel thickness, which the electrons must penetrate. Details of the level of penetration required for various molt stages of the rice weevil, have been published by this group (*1*).

There is extensive literature on radiation disinfestation of grain employing both gamma-rays and high energy electrons (*8, 9*). Cornwell (*10*) in the U.K. published studies based upon ^{60}Co gamma rays, which have provided valuable comparative information on the effects of irradiation on many of the insects listed in Table I.

Table II. Mean Insect Mortality vs. Exposure Time and Temperature for 200ppm Phosphine Fumigation (*6*)

		8h exposure		*24h exposure*	
Species	*Life Stage*	*5°C*	*32°C*	*5°C*	*32°C*
R. dominica	Eggs	45	81	57	94
	Pupae	24	98	83	100
	Adults	35	100	83	100
S, oryzae	Eggs	4	60	16	99
	Pupae	0	87	60	99
	Adults	38	100	93	100
T. castaneum	Eggs	29	79	80	100
	Pupae	3	100	44	100
	Adults	79	100	97	100

NOTE: Units are percent mortality

Colonies of the insects under study in this program were established under USDA-GMPRC guidance at the entomology laboratory at the University of Massachusetts, Amherst, MA. Full controls were used to assess population density in the grain, insect mortality and effects of product handling (*11*).

Because of the relatively low doses used in this work, (200-1600 Gy), one can operate the EB fluidized bed at high velocities (to 30 m/s). As a result, it is important to evaluate mortality due to impact alone, without the electron beam, for each life stage. For the curved transport duct shown in Figure 2, the adult *R. dominica* life stages, mortality rose to 50%. For the relatively straight transport duct shown in Figure 3 used in the 500kV system, there was 100% survival for the winter wheat control samples treated at zero Gy (impact only) showing no impact effect for all life stages. Mortality for *R. dominica* adults processed through the curved transport path of the pilot of Figure 2 was 30% which, as expected, is the life stage most vulnerable to impact mortality.

Dosimetry for these systems is conducted using radiochromic films (*12*) of 1cm^2 x 50 μm thickness. A small number of films, typically 6, are fed with the product as it is processed and the films are then recovered for assay. Change in film optical density is proportional to dose and these data are traceable to national standards using ^{60}Co gamma calibration of the film. For the dose data reported here, we have shown good

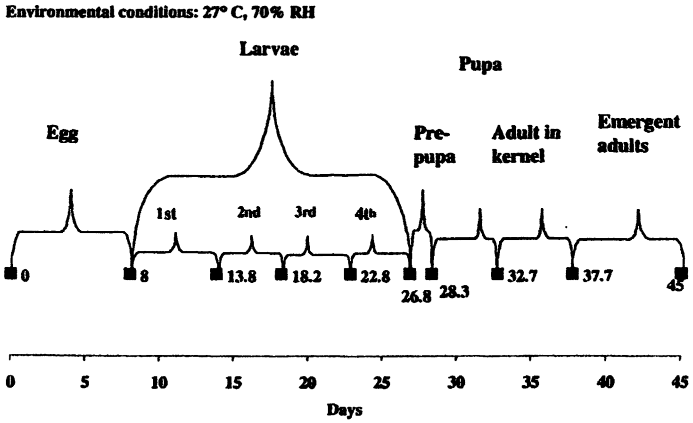
Environmental conditions: 27° C, 70% RH
Larvae
Pupa
Egg
Pre-pupa
Adult in kernel
Emergent adults
1st
2nd
3rd
4th
0
8
13.8
18.2
22.8
26.8
28.3
32.7
37.7
45
0
5
10
15
20
25
30
35
40
45
Days

Figure 4. Life Stages of Rhyzopertha dominica

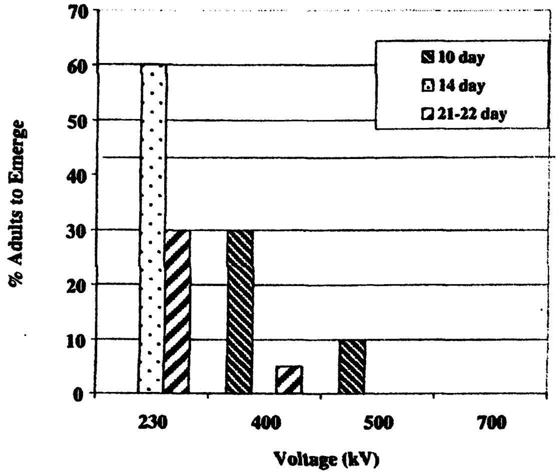

Figure 5. Larval mortality as a Function of Energy

reproducibility and an accuracy of ± 15% for the average dose delivered to material transported in the fluidized bed. The doses reported here are average values determined in this manner. Once the "longitudinal" yield value (k, in kGy.m/min/mA) for the system was determined, product velocity could then be calculated from the experimental dose determination. These values were later confirmed with actual measurements of the fluidized bed stream velocity, providing good confirmation of our dose measuring technique.

Because of the extended evaluation period for these insects after irradiation (up to 8 weeks) and the necessity of having all life stages from a colony available for treatment on the same day, our experiments were conducted at roughly two-month intervals. Triplicate samples were used with each dose: energy combination, usually with nine life stages prepared from eggs to mature adults. Throughout the extended storage period, the jars of insects/wheat were held in an incubator at 30°C and 70% R.H.

Results

Data collected from three of the 9 life-stage trials are presented in Figures 6-8 for a 7-week study of the survival of 20, 31 and 44-day-old (post oviposition) *R. dominica*. The EBFB treatment was conducted at time 0, and the emergence and mortality of adults were determined over the next two counts (1 and 2). For the earlier life stages (e.g. eggs to pupae) the adults were removed 1 and 2 weeks after emergence (45 and 52 days post oviposition), and the totals were recorded. The mortality of these adults was then followed for 5 weeks. The behavior of the controls is shown as the upper curve in each figure. At 500 kV, the 800 Gy and 1600 Gy results are presented along with some of the results recorded by Pendlebury et al. (*13*) in Figures 7 and 8, with ^{60}Co studies on *R. dominica* at 200 Gy. The data show quite good agreement of the survival curves for the two processes with a similar two-week plateau in mortality after treatment, the slope of which is dose, and possibly dose rate, dependent.

In view of the extended survival of adults irradiated during late life stages, the question immediately arises as to their fecundity and fertility during this period. Are these adults able to deposit viable eggs before mortality? Pendlebury et al. (*13*) noted that the mortality response of *R. dominica* to gamma radiation was much extended compared to other species. For pupae and adults treated at 200 Gy, the insects continued to die up to 7 to 9 weeks after irradiation, respectively. Their studies of fertility showed no delay in the effects of irradiation. Some progeny survived at doses up to 110 Gy with a 99.9% reduction in progeny, from both life stages, at that dose. A ^{60}Co dose of 160 Gy was reported as the sterilizing dose for *R. dominica*.

The trials conducted here for progeny evaluation employed the following procedure. Live adults were removed from the treated sample at one and two weeks post treatment and placed in the progeny jars (with triplicate samples). A limit of 50 adults per wheat-filled 16-ounce jar was imposed. These adults were then allowed to oviposit for up to 2 weeks and then removed from the progeny jar. They (the adults) were then transferred to a survival jar for 6 weekly assays of the number of adults surviving. The progeny jars then remained in the incubator for 6 additional weeks and any live progeny were recorded at the end of this period.

The reason why this progeny assessment is implemented is illustrated in the *R. dominica* emergence curve shown in Figure 9. This characteristic behavior at 30°C x 70% R.H. shows an emergence peak at 38 days post oviposit with completion of colony emergence some 11 days later (49 days) after oviposition. Hence, the samples were carried to the 49 days (7 weeks after the introduction of the emergent adults) age noted above in order to ensure complete yield of the emerging insects.

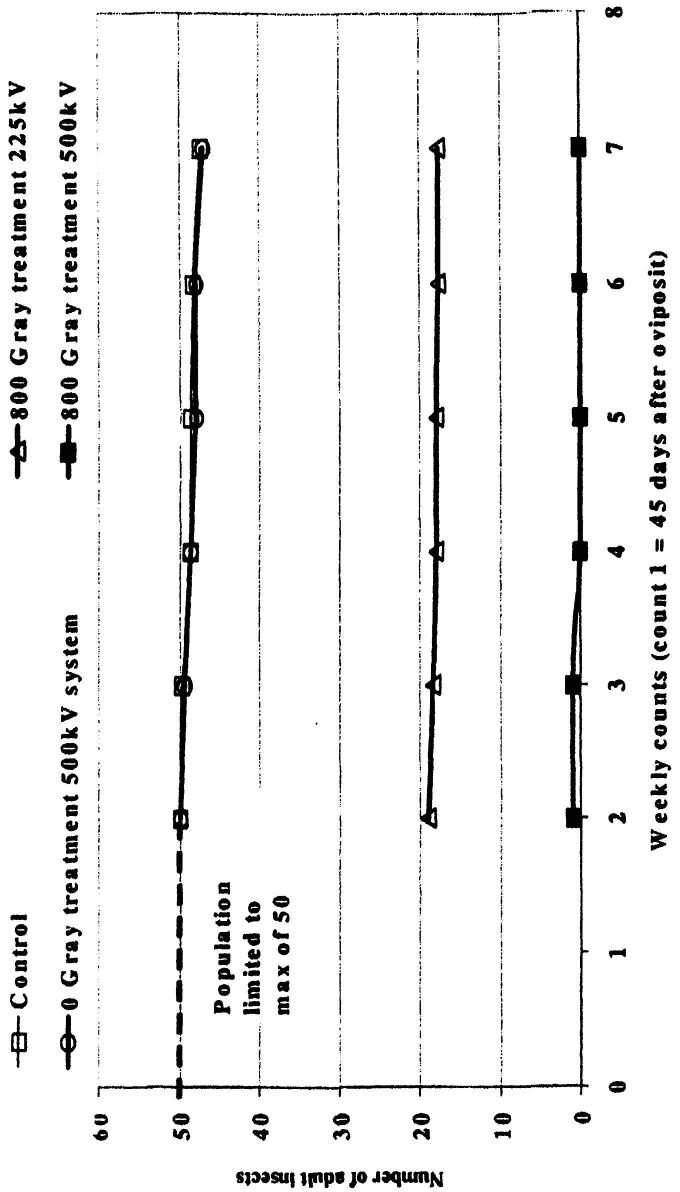

Figure 6. Survival of 20/21-day-old R. dominica at 800 Gy

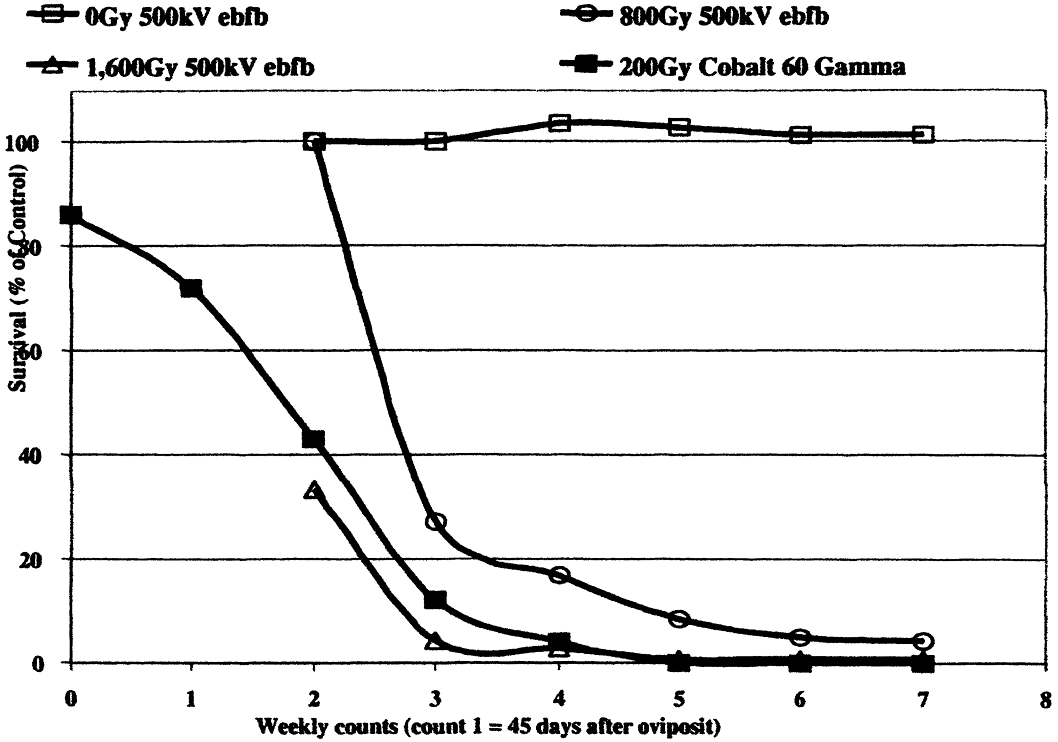

Figure 7. Survival of 31-day-old R. dominica at 800 and 1600 Gy at 500 kV (and at 200 Gy with ^{60}Co)

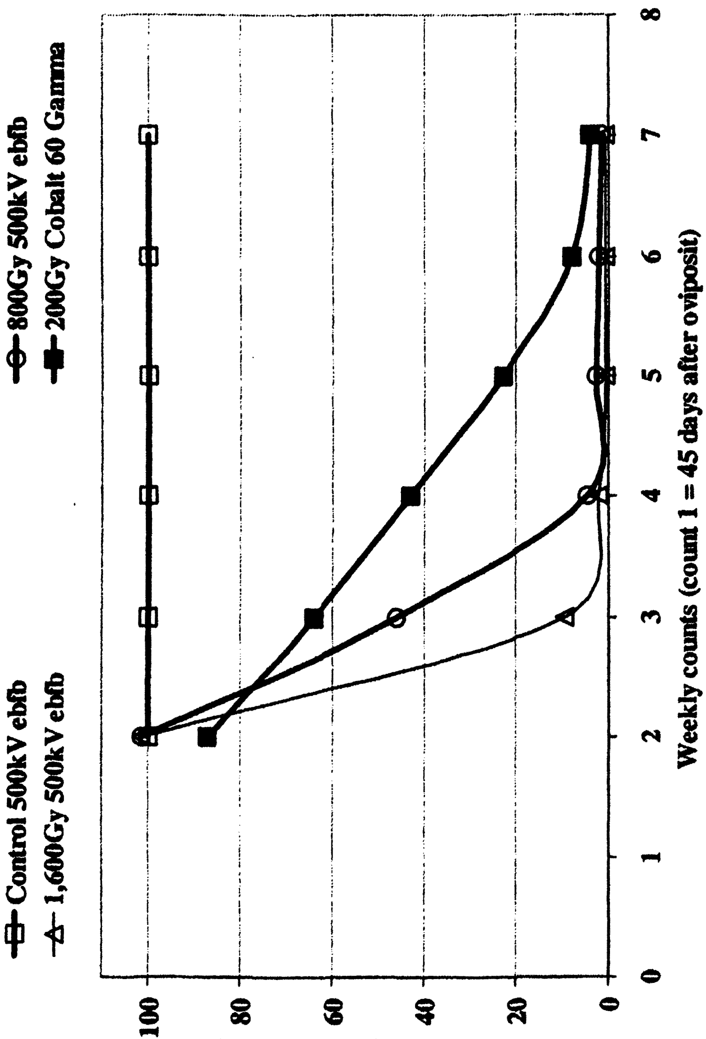

Figure 8. Survival of 44-day-old R. dominica at 800 and 1600 Gy at 500 kV (and at 200 Gy with ^{60}Co)

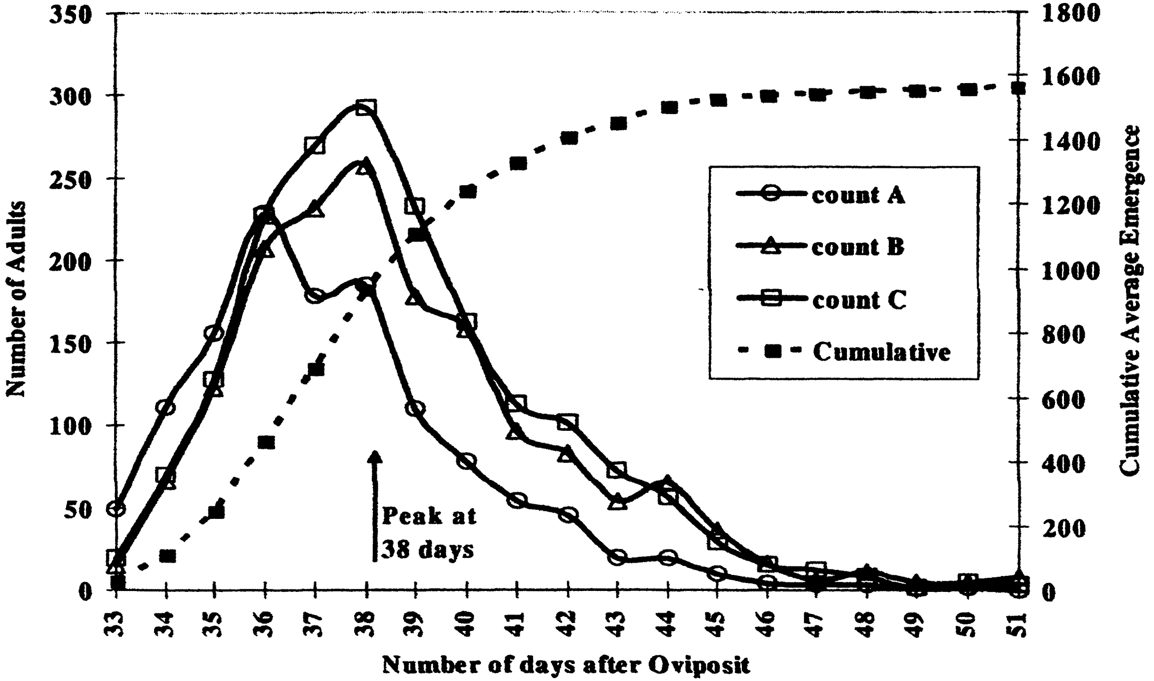

Figure 9. Rhyzopertha dominica Emergence (March 1-April 20, 2001)

The fecundity of the surviving insects is plotted as the upper curve in Figure 10 for the control, and by the two lower curves for 225 kV and 500 kV samples after 800 Gy treatments, respectively. These experiments revealed elevated progeny yields from the older life stages by as much as a factor of 5 for the adult stages.

As shown in Figure 10, at 225 kV x 800 Gy, some progeny were recorded for insects irradiated during the deeply embedded third instar at 20 d and for the pupae and emerging adults at 30-45 d. The decreased fecundity of the insects is not as prominent at 225 kV. For the 500 kV treatment at the same 800 Gy dose, no progeny were observed for all life stages.

Growth Model Predictions

At the completion of the experimental studies, the effects of irradiation on each of the life stages, including post-irradiation female fecundity, were evaluated with the use of a previously described (*14*) farm bin simulation model. A well-tested insect model was used to predict daily development and population growth of ***Rhyzopertha dominica*** as a function of grain temperature and moisture. The EBFB data on the survivorship for each life stage was then added to the model. Simulations were started on June 1, (day 152) with 50 adults at an initial grain temperature of 30°C. The wheat moisture content was 12%. The insect population behavior of the infested sample with no intervention is predicted up to 215 days in Figure 11, while the effects of EBFB treatment at 45 days (day 197) at 225 kV and 500kV are illustrated in Figures 12 and 13, respectively.

Discussion

The trials described here have addressed the limitations of the fluidized bed process detailed in earlier publications (*1*, *7*). The use of 500 kV electrons has been shown to be efficacious for all life stages of *R. dominica* in winter wheat. Although 1600 Gy doses were used for comparative purposes, it is clear from this work that doses of 1 kGy or less are adequate with penetration capable of reaching the most heavily protected larval stages for this internal feeder. The progeny studies have shown that eggs laid during the short-term survival of adults are infertile. This has been an important result of the study in completing the efficacy assessment of the physical process. The integrated effect is illustrated by the population predictions of the model shown in Figure 13.

The economics (2) of the EBFB process compared with those of fumigation appear to be attractive particularly given the improved human and food safety possible by this physical technique. Using the *R. dominica* simulation model projections and the mortality data generated here, it will now be possible to evaluate EBFB applications with various cereal grains (wheat, corn, rice, and barley) on which chemical treatments are now so widely used.

Acknowledgements

The contributions of Jeremy Pariseau to the development of the experimental hardware, to the execution of the experiments and in the reduction of the data, are acknowledged. The careful insect assays and colony care by Derek Sturtevant at U.MA/Amherst, and the continuing entomology guidance of Reg Coler and Andy

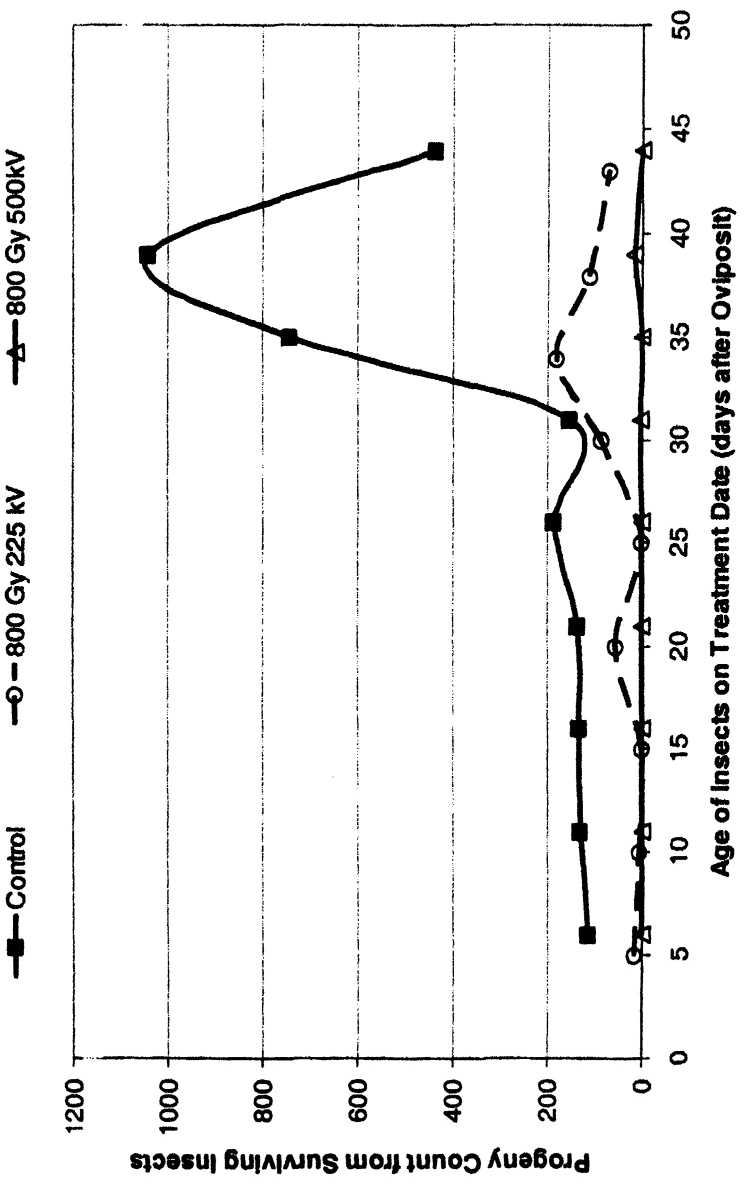

Figure 10. Progeny Counts for R. dominica EBFB Treated at 800 Gy

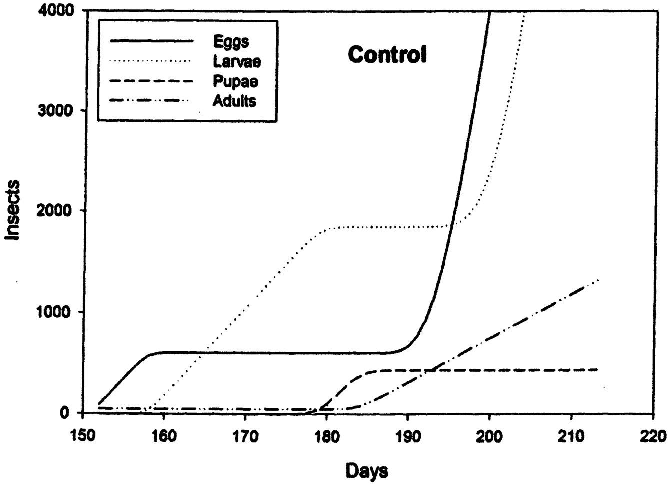

Figure 11. R. dominica Population Predictive Model with No Intervention (Initial Population = 50 adults)

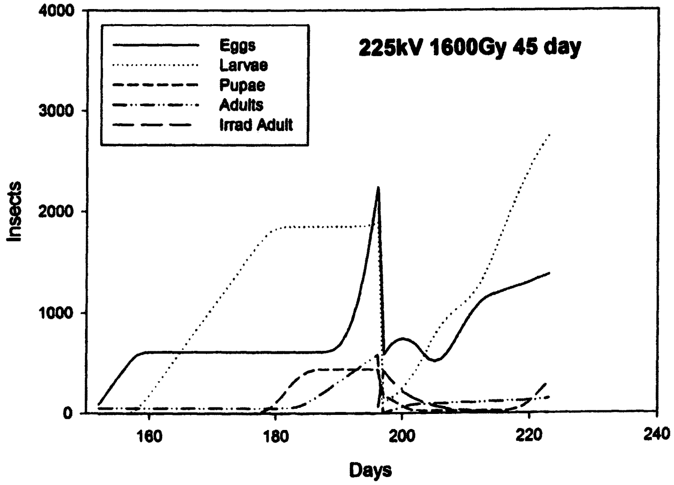

Figure 12. R. dominica Population Predictive Model with ebfb Treatment

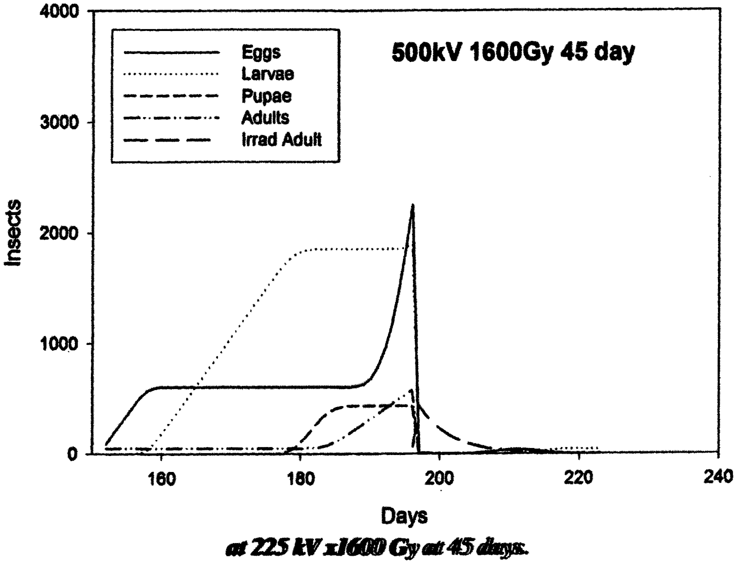

Figure 13. R. dominica Population Predictive Model with EBFB Treatment at 500 x1600 Gy at 45 days.

Slocombe at the Amherst campus of the University have been essential to the success of this program. This work has been supported by USDA, SBIR Grant # 00-33610-9471.

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October 23, 2009 | http://pubs.acs.org
Publication Date: January 2, 2004 | doi: 10.1021/bk-2004-0875.ch004

Chapter 4

Mechanisms and Prevention of Off-Odor Production and Color Changes in Irradiated Meat

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Irradiation is the best-known intervention strategy that can ensure safety of raw meat. However, irradiation can produce a characteristic aroma, accelerate lipid oxidation and change the color in meat. A number of meat processors is currently marketing irradiated ground meat products. Irradiated meat products can develop a characteristic odor described as "barbecued corn-like" or "bloody sweet" odor. The mechanisms and sources of off-odor production in irradiated meat indicates that volatiles responsible for the off-odor are sulfur-containing compounds such as methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide; all of which were generated by the radiolytic degradations of sulfur-containing amino acids present in meat. These sulfur compounds were highly volatile and could be eliminated by storing the irradiated meat under aerobic conditions. Irradiation accelerates lipid oxidation in meat only under aerobic conditions, but the types and amounts of volatiles produced by irradiation do not correlate well with the degrees of lipid oxidation. The pigment responsible for pink color in irradiated turkey breast is a carbon monoxide-myoglobin (CO-Mb) complex, and the changes in oxidation-reduction potential (ORP) in meat played an important role in the formation of CO-Mb. Irradiated meat produced a significant amount of CO

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

while the ORP of meat decreased after irradiation. Most chemical changes in irradiated meat are associated with free radical reactions, and the resultant sulfur compounds could be controlled by using appropriate packaging methods. Appropriate packaging methods and additive combinations, therefore, can be used to control off-color, off-odor, and lipid oxidation in irradiated raw meat during storage. The effects of double packaging and additive combinations on the control of off-odor volatiles, off-color, and lipid oxidation in irradiated meat are discussed.

Introduction

Meat and poultry are primary sources of food-borne pathogens. Based on data of the Centers for Disease Control and Prevention, food-borne illnesses account for estimated 76 million cases, 325,000 hospitalizations, 5,000 deaths, and $6.7 billion in human medical and productivity losses annually (*1*). In addition, the US Public Health Service has targeted reducing the number of human cases caused by each of these food-borne pathogens significantly by the year 2010.

Irradiation is among the best-known methods for control of potentially pathogenic microorganisms in raw meat, but its application in poultry is limited because of quality and health concern in association with consuming the irradiated meat. Irradiation can produce a characteristic aroma, and changes in meat flavor and color that could affect consumer acceptance. The formation of a pink color and off-odor is a critical issue for the use of irradiation with broiler breast meat because consumers associate the presence of pink color in raw and cooked breast meat with contamination or undercooking, and the off-odor and off-flavor with undesirable chemical reactions. As a result of these consumer perceptions, the poultry meat industry has not adopted irradiation to achieve its food safety benefits. The government has made efforts in supporting research and consumer education to establish that irradiation is a safe process, and irradiation does not significantly change nutritional compositions in raw or cooked meat. However, when consumers find an unusual color or odor/flavor changes in a familiar meat or meat product, this may cast unnecessary doubts in their minds as to what other changes may have occurred. Therefore, methods that can control the off-odor in raw meat, and the off-flavor, off-color (pinking) and lipid oxidation in cooked meat are important for increased consumer acceptance of irradiated meat.

Quality Changes in Meat by irradiation

A. Lipid Oxidation

Lipid oxidation mechanisms in irradiated meat are not fully understood, but they are likely to be similar to those in non-irradiated meat. Ionizing radiation generates hydroxyl radicals in aqueous (*2*) or oil emulsion systems (*3*). Irradiation can produce hydroxyl radicals which could generate lipid oxidation-induced off-odor in meat because muscle cells consist of 75% or more water. Irradiation-induced chemical changes are dose dependent, and the presence of oxygen has a significant effect on the development of oxidation and odor intensity (*4, 5*). Ahn et al. (*6*) reported that the TBARS values of vacuum-packaged patties irradiated at 1.5, 3.0 and 4.5 kGy doses and stored at 4°C were not much different from those of nonirradiated (control) at each storage time. The TBARS values increased sharply during refrigerated storage in aerobic packaging, but the effect of irradiation was not found at 2 weeks of storage (Table I). This result agreed with the previous work (*7*), suggesting that oxygen availability was more important for the development of lipid oxidation than the irradiation.

Table I. TBARS of Vacuum- and Aerobically Packaged Raw Pork Patties Irradiated and Stored at 4°C or -40°C

	Vacuum packaging				*Aerobic packaging*			
Storage	*0 kGy*	*1.5 kGy*	*3.0 kGy*	*4.5 kGy*	*0 kGy*	*1.5 kGy*	*3.0 kGy*	*4.5 kGy*
	TBARS (mg malondialdehyde/kg meat)							
Storage at 4°C								
0 wk	0.08^{c}	0.08^{c}	0.09^{b}	0.10^{b}	0.08^{by}	0.07^{cy}	0.11^{cxy}	0.12^{cx}
1 wk	0.22^{a}	0.21^{a}	0.24^{a}	0.32^{a}	0.34^{a}	0.45^{b}	0.43^{b}	0.43^{b}
2 wk	0.14^{b}	0.12^{b}	0.15^{b}	0.16^{b}	0.40^{a}	0.85^{a}	0.65^{a}	0.82^{a}
	0 kGy	*2.5 kGy*	*5.0 kGy*	*7.5 kGy*	*0 kGy*	*2.5 kGy*	*5.0 kGy*	*7.5 kGy*
Storage at -40°C								
0 mo.	0.15^{y}	0.18^{xy}	0.21^{xy}	0.23^{ax}	0.15^{z}	0.19^{az}	0.29^{y}	0.39^{ax}
1.5 mo.	0.16	0.18	0.18	0.20^{ab}	0.15^{y}	0.21^{ay}	0.28^{x}	0.32^{abx}
3 mo.	0.13	0.14	0.15	0.12^{b}	0.11^{z}	0.12^{bz}	0.24^{y}	0.26^{bx}

[a-c] Means with different letter within a column is significantly different ($P < 0.05$).
[x-z] Means with different letter within a row with the same packaging method is significantly different ($P < 0.05$). TBARS: 2-thiobarbituric acid reactive substances.
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Luchsinger et al. (*8*) showed that TBARS values of both chilled and frozen boneless pork chops were relatively stable, regardless of display day, dose and irradiation sources. Our study with frozen meat also indicated that the TBARS of vacuum packaged and irradiated pork patties was not significantly different than that of nonirradiated (control) after 1.5 and 3 months of storage. In aerobic-packaged irradiated patties, however, the TBARS of meat increased with increased irradiation dose but storage time had little effect (Table I). This result indicates that the radiation chemistry of refrigerated and frozen meat could be different. Tarte (*9*) reported that temperature has significant effects on the formation of radiolytic products, and that the reactive intermediates of water radiolysis were trapped in deep-frozen materials and thus were kept from reacting with each other or with the substrates. During the warming process, however, they tend to react with each other rather than with the substrates (*10*).

Lipid oxidation was a significant problem in irradiated meat only when it was irradiated and stored under aerobic conditions (*4, 11*). Without oxygen, lipid oxidation in cooked meat did not progress even with added prooxidants. In the presence of oxygen, ferrous iron was the strongest prooxidant in both raw and cooked meats. Excluding oxygen from meat and meat products could protect them from oxidation almost completely by blocking the initiation step of the chain reaction (*12, 13, 14*). Effects of raw-meat packaging, irradiation and cooked-meat packaging on lipid oxidation of cooked pork patties during storage were compared (Table II). In cooked patties, TBARS values at Day 0 were not influenced by packaging or irradiation conditions of raw meat. After 3 and 7 days of storage, TBARS values of cooked meat with vacuum packaging (A-C-V, A-IR-V, and V-IR-V) remained unchanged or slightly increased, but those with aerobic packaging (A-C-A, A-IR-A, and V-IR-A) increased by 6 to 9 fold from the 0-day values. The pork patties cooked 3 days after irradiation had higher TBARS values than those cooked 2 h after irradiation, and continued to have higher TBARS throughout storage. This indicated that the initial oxidation status of cooked meat was determined by the degree of lipid oxidation in raw meat before cooking. Also, significant amounts of primary and secondary lipid oxidation by-products, which influenced TBARS of cooked meat, had formed in raw meat during storage before cooking. Therefore, the baseline lipid oxidation status of raw meat was a very important determinant for the progression of lipid oxidation in cooked meat. The exposure to oxygen was also important for the oxidation of cooked meat during storage. As shown in previous reports (*12, 13*), preventing exposure to oxygen after cooking was more important than packaging, irradiation, or storage of raw meat for maintaining low TBARS values.

Table II. Effect of Raw-Meat Packaging, Irradiation, and Cooked-Meat Packaging on Lipid Oxidation of Cooked Pork Patties[1]

	A-C-A[*]	*A-C-V*	*A-IR-A*	*A-IR-V*	*V-IR-A*	*V-IR-V*
	TBARS (mg MDA/kg meat)					
0 day storage after cooking[2]						
0 day after IR[3]	0.26^{b}	0.19^{b}	0.34^{b}	0.26^{b}	0.32^{b}	0.20^{b}
3 days after IR	0.61^{a}	0.61^{a}	0.67^{a}	0.67^{a}	0.59^{a}	0.59^{a}
3 days storage after cooking						
0 day after IR	2.46^{bx}	0.32^{bz}	2.83^{bx}	0.34^{bz}	1.68^{by}	0.36^{bz}
3 days after IR	5.34^{ax}	0.71^{az}	4.85^{ax}	0.66^{az}	4.11^{ay}	0.79^{az}
7 days storage after cooking						
0 day after IR	3.48^{bx}	0.44^{bz}	3.44^{bx}	0.39^{bz}	2.44^{by}	0.45^{bz}
3 days after IR	5.46^{ax}	0.81^{ay}	5.88^{ax}	0.79^{ay}	5.47^{ax}	0.75^{ay}

[1]Raw-meat patties were irradiated at 0 or 4.5 kGy dose (ave.). [2]Samples were analyzed within 1 hr after cooking, [3]Storage of raw meat before cooking, 0 day after IR samples were stored 2 hr after irradiation. [x-z]Different letters within a row are significantly different ($P < 0.05$). [a,b]Values with different superscript letters within a column of the same storage time after cooking are different ($P < 0.05$). n=12.

[*]Abbreviation of treatments: A, aerobic packaging; V, vacuum-packaging; C, control, nonirradiated; IR, irradiated at 4.5 kGy. MDA, malondialdehyde.

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Diehl (*10*) indicated that the irradiation of aqueous systems produced hydrogen peroxide, particularly in the presence of oxygen. During post-irradiation storage, hydrogen peroxide gradually disappears while other constituents of the system are oxidized. Some oxidized compounds, absent or present at lower concentrations immediately after irradiation, can increase hours or days after irradiation. One study with an oil emulsion system showed that TBARS values of irradiated emulsion samples immediately after irradiation were lower than those of nonirradiated samples (*15*). After 10 days of storage at 4 °C, however, irradiated samples developed higher TBARS values than nonirradiated emulsions. Arachidonic acid, linolenic acid, and fish oil, which have a high proportion of multi-double-bonded fatty acids, had accelerated lipid oxidation after irradiation (Table III). Shahidi and Pegg (*16*) reported that aldehydes contributed the most to oxidation flavor and rancidity in cooked meat, and hexanal was the predominant volatile aldehyde found. Among the volatiles of emulsion prepared with arachidonic acid, linolenic acid, or fish oil, aldehydes increased the most during storage (Table III). Hexanal was produced only in emulsions prepared from arachidonic acid and propanal only from linolenic acid, indicating that n-3 PUFAs are the source of propanal and n-6 PUFAs of hexanal. Fish oil that contains high amount of n-3 PUFAs produced very large amounts of propanal. Longer storage time increased the amount of aldehydes and TBARS values in these oil emulsions, but irradiation had minimal effect on the increase of aldehydes and TBARS. Also, volatiles from lipids accounted for only a small part of the off-odor in irradiated samples. In summary, irradiation increased lipid oxidation of meat under aerobic conditions. However, oxygen played a more important role on the development of lipid oxidation in meat than irradiation, especially in cooked meat.

Table III. Production of Aldehydes and TBARS Value in Emulsions Prepared with Arachidonic and Linolenic Acid, and Fish Oil

	Arachidonic acid		*Linolenic acid*		*Fish oil*	
Volatiles	*0 kGy*	*5 kGy*	*0 kGy*	*5 kGy*	*0 kGy*	*5 kGy*
	total ion counts x 10^4					
Day 0						
2-Propenal	0	0	0	0	179	287
Propanal	0	0	0	1519	0	0
Butanal	0	0	0	0	465	175
Pentanal	0	0	0	0	323	81
Hexanal	0	0	0	0	0	0
Total aldehydes (%)	0	0	0	0.5	5.3	7.8
TBARS (mg/1000g)	2.58	1.41	4.51	1.27	2.27	2.38
Day 10						
2-Propenal	11435	27531	4426	3393	7070	2775
Propanal	794	0	32297	30809	24403	10899
Butanal	0	223	117	0	1314	455
Pentanal	1180	2494	0	0	580	248
Hexanal	28864	58702	0	0	0	0
Total aldehydes (%)	33.2	47.9	9.5	6.8	79.4	87.1
TBARS (mg/kg oil)	143.43	140.10	103.68	76.37	54.26	26.86

n = 4. SOURCE: Reproduced with permission from reference 15. Copyright 2003 J. Food Sci.

B. Sources and Mechanisms of Off-Odor Production in Meat

Meat products irradiated in the non-frozen state develop a characteristic and readily detectable, aroma. Hashim et al. (*17*) described the irradiation odor as a "bloody and sweet" aroma, and Ahn et al. (*6*) described it as a "barbecued corn-like" odor. Sensory panels clearly detected an irradiation odor from irradiated pork patties at Day 0, but could not separate irradiation dose effect in both vacuum- and aerobically packaged patties. Irradiated vacuum-packaged patties maintained irradiation off-odor during 2-weeks of storage, but the intensity of irradiation off-odor in aerobically packaged pork disappeared after 1 week or longer of refrigerated storage. This indicated that packaging plays a very important role on the odor of irradiated meat.

Volatiles of Aerobically Packaged Meat

Irradiation had a significant impact on the amount and profile of volatiles in meat. The volatiles in irradiated meat were determined using a dynamic headspace GC/MS method. At Day 0, aerobically packaged irradiated pork produced a greater number of volatiles than the nonirradiated pork (Table IV). Butane, propane, mercaptomethane, dimethyl sulfide, methyl thioacetate and dimethyl disulfide, not detected in nonirradiated pork, were produced in irradiated pork.

Among the volatiles produced by irradiation, mercaptomethane, dimethyl disulfide, methyl thioacetate and dimethyl disulfide (sulfur-containing volatile compounds) were the major ones. Carbon disulfide was found in both irradiated and nonirradiated pork, but its concentration increased significantly after irradiation. Hexanal, an off-flavor volatile typically associated with oxidative changes to linoleic acid, was detected only in aerobically packaged meat. Most sulfur and carbonyl compounds have low odor thresholds and were considered to be important to irradiation odor (*18*). Batzer and Doty (*19*) reported that methyl mercaptan and hydrogen sulfide were important to irradiation odor. Patterson and Stevenson (*20*) found that dimethyl trisulfide was the most potent off-odor compound, followed by *cis*-3- and *trans*-6-nonenals, oct-1-en-3-one, and bis (methylthio-) methane in irradiated chicken meat. Dimethyl trisulfide was detected in irradiated pork loin in one previous work (*21, 22*), but no noticeable amount of dimethyl trisulfide was found in another study (23). This indicated that the sulfur-containing compounds could be the major volatile components responsible for the characteristic odor in irradiated pork, and supports the concept that the changes that occur following irradiation are distinctly different from those of the warmed-over flavor of oxidized meat.

After 10 days of storage under aerobic packaging condition, the amount of total volatiles in pork decreased by 30% to 60%. As in 0-day pork, mercaptomethane, dimethyl sulfide, and methyl thioacetate were found only in irradiated pork. Propane and dimethyl disulfide, found in irradiated meat at Day 0, were not detected after 10 days of storage under aerobic conditions. The amounts of other volatiles including acetaldehyde, mercaptomethane, furan and ethanol significantly decreased after 10 days of storage, but the decrease of mercaptomethane was the largest (Table IV). The content of dimethyl sulfide

Table IV. Relative Production of Volatiles of Aerobically Packaged Pork *L. dorsi* Muscle at 0 Days of Storage at 4°C

Volatile	*0 day*		*10 days*	
compound	*0 kGy*	*4.5 kGy*	*0 kGy*	*4.5 kGy*
	-------------- Peak area (pA x sec) x10^4 --------------			
Butane	0[b]	136[a]	38[b]	80[a]
Acetaldehyde	962	506	197	235
Propane	0[b]	87[a]	0	0
Mercaptomethane	0[b]	3024[a]	0	37
Pentane	321[b]	581[a]	246	524
Furan	41	72	26	33
Ethanol	956[a]	233[b]	64	112
Dimethyl sulfide	0[b]	682[a]	0[b]	749[a]
Carbon disulfide	185[b]	422[a]	139	216
3-Methyl pentane	31	48	50	64
1-Hexene	29[b]	74[a]	22	22
Hexane	170[b]	323[a]	224	199
1-Propanol	117[a]	19[b]	15	21
Diacetyl	323[a]	43[b]	76	73
2-Butanone	283[b]	513[a]	217[b]	379[a]
3-Methyl butanal	28	45	21	27
1-Heptene	26[b]	125[a]	39	49
Heptane	148[b]	284[a]	121	145
2-Pentanone	93[a]	0[b]	166[a]	0[b]
Methyl thioacetate	0[b]	402[a]	0[b]	194[a]
Pentanal	74	103	35	45
Dimethyl disulfide	0[b]	612[a]	0	0
1-Octene	139	112	46	53
Octane	1226	1305	459	745
2-Octene	32	32	14	18
3-Octene	5[b]	31[a]	13	18
Hexanal	16	16	42	104
Nonane	37	32	30	38
Total volatiles	5242[b]	9862[a]	2300[b]	4180[a]

[a-d] Different letters within a row are different ($P < 0.05$). n = 4.

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was the only sulfur compound that increased significantly after 10 days of storage under aerobic conditions. This result suggests that some of these compounds escaped from the packaging bags and others were converted to other compounds via chemical or enzymatic reactions during storage.

Volatiles of Vacuum-Packaged Meat

Volatile profiles of vacuum-packaged pork at Day 0 (Table V) were relatively similar to those of the aerobically packaged pork except for minor differences. The changes of volatiles in pork after 10 days of storage in vacuum packaging were different from those in aerobic packaging. Total volatile content of nonirradiated pork decreased but that of the irradiated pork increased significantly. Unlike in aerobically packaged pork, the amounts of all sulfur-containing volatile compounds increased significantly. Among these sulfur compounds, the increase of dimethyl sulfide in irradiated pork during the 10-day storage was the largest with approximately 6 fold increase from the Day 0.

The production of volatiles was strongly influenced by packaging and irradiation (Table VI). Storage time had less effect than meat condition and irradiation on the content of many volatile compounds. Among the volatiles found in irradiated and nonirradiated pork, the production of 3-pentanol, 3-methyl pentane, 2-butanone, 1-octene, octane, 3-octene and hexanal was influenced by irradiation; the formation of pentane, ethanol, dimethyl sulfide, carbon disulfide, 1-propanol, 3-methyl butanal, heptane, methyl thioacetate, dimethyl disulfide and total volatiles was influenced by storage time. Butane, propane, mercaptomethane, dimethyl sulfide, hexane, heptane, 2-octene, and hexanal in pork were influenced by packaging methods. Irradiation was the only factor that influenced the production of all five sulfur-containing volatile compounds in pork. The production of hexanal, a major volatile related to oxidative changes in meat, was not influenced by irradiation but by storage and packaging methods.

In summary, irradiation increased the production of sulfur-containing volatiles (carbon disulfide, mercaptomethane, dimethyl sulfide, methyl thioacetate and dimethyl disulfide), but not lipid oxidation products in pork, regardless of packaging conditions. Most of the sulfur-containing volatiles produced in meat by irradiation escaped during storage under aerobic packaging conditions. Irradiation and storage of meat in vacuum packaging may be desirable for a long-term storage but may reduce the acceptance of irradiated meat because of the sustaining off-odor.

Study with Amino Acids and Amino Acid homopolymers

Our earlier studies on the off-odor production in irradiated meat have mainly focused on lipid oxidation products. However, irradiation off-odor was

Table V. Relative Production of Volatiles of Vacuum-Packaged Pork *L. dorsi* Muscle during Storage at 4°C

Volatile	*0 day*		*10 days*	
compound	*0 kGy*	*4.5 kGy*	*0 kGy*	*4.5 kGy*
	---------------- Peak area (pA x sec) x10^4 ----------------			
Butane	0^b	96^a	0^b	331^a
Acetaldehyde	1261^a	326^b	272	367
Propane	0^b	86^a	0^b	111^a
Mercaptomethane	0^b	2185^a	0^b	1692^a
Pentane	235^b	419^a	205^b	637^a
Furan	54	77	13^b	29^a
Ethanol	761^a	104^b	269	397
Dimethyl sulfide	0^b	759^b	0^b	4877^a
Carbon disulfide	0^b	189^a	211^b	292^a
3-Methyl pentane	0	0	110	104
1-Hexene	0^b	53^a	0^b	89^a
Hexane	115^b	183^a	130^b	290^a
1-Propanol	43^b	16^b	130^a	68^b
Diacetyl	204^a	0^b	123^a	63^b
2-Butanone	201	120	346^b	760^a
3-Methyl butanal	17	41	16^b	91^a
1-Heptene	14	97	0^b	191^a
Heptane	95^b	178^a	65^b	273^a
2-Pentanone	46^a	0^b	329^a	0^b
Methyl thioacetate	0^b	187^a	0^b	410^a
Pentanal	46	57	31^b	79^a
Dimethyl disulfide	0^b	239^a	0^b	358^a
1-Octene	119^b	328^a	30^b	141^a
Octane	918	1089	718	880
2-Octene	13	28	0^b	30^a
3-Octene	0^b	20^a	0^b	25^a
Hexanal	0	0	22^b	128^a
Nonane	0^b	39^a	42	58
Total volatiles	4142^d	6916^a	3062^b	12771^a

$^{a-d}$Different letters within a row of same storage time are different ($P < 0.05$). n = 4.
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Table VI. Statistical Significance of Effects of Meat Condition, Irradiation Dose, Storage Time and Packaging on Volatile Production from Pork *L. dorsi* Muscle

Volatile compound	*Irradiation*	*Storage*	*Packaging*
	Probability		
Butane	0.0001	0.0001	0.0004
Acetaldehyde	0.0001	0.0001	0.12
Propane	0.0001	0.0001	0.0001
Mercaptomethane	0.0001	0.001	0.01
Pentane	0.0001	0.88	0.43
Furan	0.0002	0.0001	0.77
Ethanol	0.0001	0.25	0.65
Dimethyl sulfide	0.0001	0.09	0.02
Carbon disulfide	0.01	0.24	0.07
3-Methyl pentane	0.62	0.0001	0.05
1-Hexene	0.002	0.001	0.15
Hexane	0.0001	0.004	0.0001
1-propanol	0.0001	0.57	0.21
Diacetyl	0.0001	0.03	0.0001
2-Butanone	0.13	0.004	0.14
3-Methyl butanal	0.0001	0.38	0.41
1-Heptene	0.0001	0.0001	0.93
Heptane	0.0001	0.17	0.0008
2-Pentanone	0.0001	0.006	0.81
Methyl thioacetate	0.0001	0.31	0.52
Pentanal	0.03	0.003	0.05
Dimethyl disulfide	0.0001	0.08	0.99
1-Octene	0.05	0.0009	0.05
Octane	0.11	0.007	0.47
2-Octene	0.0001	0.02	0.01
3-Octene	0.49	0.02	0.80
Hexanal	0.20	0.0001	0.006
Nonane	0.002	0.0001	0.22
Total volatiles	0.0001	0.99	0.56

n=48. SOURCE: Reproduced with permission from reference 23. Copyright 2001 Elsevier.

not related to lipid oxidation-dependant volatiles. Sensory results also clearly indicate that the main source of irradiation off-odor was the sulfur compounds derived from proteins, not lipids. To determine the major sources and mechanisms of off-odor volatiles by irradiation, amino acid model systems were prepared. The production of many new volatiles from amino acids upon irradiation indicated that more than one site in the amino acid side chain was susceptible to free radical attack and many volatiles were apparently produced by the secondary chemical reactions after the primary radiolytic degradation of the side chains. Only sulfur-containing volatiles, however, produced a strong odor that was similar to the irradiation odor (Table VII). The perception of odor from samples containing sulfur volatiles changed depending upon their composition and amounts present in the sample. Sulfur compounds were not only produced by the radiolytic cleavage of side chains (primary reaction), but also by the secondary reactions of primary sulfur compounds with other compounds around them. The amounts and kinds of sulfur compounds produced from irradiated methionine and cysteine indicated that methionine is the major amino acid responsible for irradiation off-odor (Table VIII). The total amount of sulfur compounds produced from cysteine is only about 0.25-0.35% of methionine, even after the proportion of cysteine or methionine in each of the dimmer, trimer or tetramers were considered. Therefore, the contribution of methionine to the irradiation odor is far greater than that of cysteine.

The odor intensity of sulfur-containing amino acids was much stronger and more stringent than that of other amino acid groups. This indicated that sulfur compounds were the most influential in irradiation off-odor, but volatiles from other amino acid groups also played an important role in overall odor perception. Sulfur compounds have very low odor thresholds and most of them are considered to be the major cause of off-odor in irradiated meat. However, some sulfur compounds, such as 2-pentylthiophene, are important for freshly cooked meat flavor (*24*).

The volatile profiles and sensory characteristics of amino acids clearly explained why irradiation odor was different from lipid oxidation odor, and why lipid oxidation was responsible for only a small part of the off-odor in irradiated meat (*11, 21, 25*). Patterson and Stevenson (*20*) identified dimethyl trisulfide and bis (methylthio-) methane as the most potent off-odor sulfur compounds in irradiated chicken meat, but our data indicated that many other sulfur compounds could be produced from methionine and cysteine (*26*). The volatility of aroma compounds depends on the vapor-liquid partitioning of volatile compounds, which determines the affinity of volatile molecules for each phase (*27*), and the interactions among food components such as carbohydrates and proteins affect the release of volatile compounds in foods (*28*). Physicochemical conditions of foods, which influence conformation of proteins, also are closely related to flavor release (*29*). Jo and Ahn (*30*) reported that the amount of volatiles

Table VII. Major Volatile Compounds from Irradiated Amino Acid Homopolymers and their Odor Characteristics

Amino acid polymer	*Major volatiles*	*Odor characteristics*
Poly-aspartic acid	2-propanone, methyl cyclopentane, toluene[a]	no odor
Poly-glutamic acid	acetaldehyde, 2-propanone	sweet, honey
Poly-alanine	acetonitrile, methyl propionate, acetaldehyde, 2-propanone	seaweed
Poly-glycine	acetaldehyde, 2-methyl propanal, 2-methyl butanal, 3-methyl propanal	seashore odor
Poly-proline	2-propanone, hexane	organic solvent
Poly-serine	acetaldehyde, 1,1-oxybis ethane, 2-propanone	cattle barn odor
Poly-threonine	acetaldehyde, 2-propanone, acetic acid ethyl ester, 2-ethoxy butane, 2,3-dihydro-1,4-dioxin, 1,4-dioxin, methyl butyrate, 1,1-oxybis ethane	Chinese herbal medicine
Poly-asparagine	methyl cyclopentane, benzene, toluene	no odor
Poly-glutamine	acetaldehyde, 1,1-oxybis ethane, 2-propanone	hospital odor
Poly-tyrosine	acetaldehyde, tetrahydrofuran, 2-methyl-1,3-dioxalane, benzene, toluene, cyclohexane, 2,3-dihydro-1,4-dioxin	seaweed or seashore
Poly-histidine	2-methoxy-2-methyl propane, toluene	sweet
Poly-lysine	acetaldehyde, propanal, butanal, 2-methyl dioxalone, benzene, 2-propanone	coleslaw, sour
Glutathione	carbon disulfide, dimethyl disulfide, methyl cyclopentane	hard-boiled eggs, sulfury
Met-Ala	acetaldehyde, mercaptomethane, dimethyl sulfide, methyl thiirane, 3-(methylthio)-1-propene, ethanoic acid- S-methyl ester, dimethyl disulfide, methyl ethyl disulfide, 2,4-dithiapentane, 2-methyl propanal	boiled eggs, sulfury rotten vegetable
Met-Gly-Met-Met	mercaptomethane, pentanal, dimethyl sulfide, (methylthio)-ethane, benzene, 1-heptanethiol, 3-(methylthio)-1-propene, ethanoic acid-S-methyl ester, dimethyl disulfide, methyl ethyl disulfide, 2-butanamine, 1,3-dimethyl benzene, 1,4-dimethyl benzene, isopropyl benzene, ethyl benzene	boiled cabbage, sulfury, rotten vegetable

[a] Volatiles written in italic did not produce detectable odor at the levels found in the samples.

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released from oil emulsion correlated negatively with fat content. The release of nonpolar hydrocarbons was not influenced, but polar compounds such as aldehydes, ketones and alcohols were greatly influenced by water. This indicated that the relative amounts of volatile compounds released from meat systems could be significantly different from those in the aqueous system tested here. However, the results from this study confirmed the sources of volatile compounds critical to irradiation off-odor reported by Jo and Ahn (*31*).

Table VIII. Production of Volatile Compounds from Sulfur-Containing Amino Acid Tetramer or Oligomers by Irradiation

Volatiles	*0 kGy*	*5 kGy*
	-------- total ion counts x 10^4	---------
Glutathione (γ-Glu-Cys-Gly)		
Carbon disulfide	0^b	589^a
Dimethyl disulfide	0^b	214^a
Met-Gly-Met-Met		
Mercaptomethane	0^b	17325^a
Dimethyl sulfide	0^b	201541^a
(Methylthio) ethane	0^b	2053^a
1-Heptanethiol	0^b	94^a
3-(Methylthio)-1-propene	0^b	122^a
Ethanthioic acid, S-methyl ester	0^b	170^a
2-Methyl-2-(methylthio) propane	92^b	149^a
Dimethyl disulfide	1430^b	351320^a
Methyl ethyl disulfide	0^b	1935^a

^{a,b}Means with no common superscript differ significantly ($P < 0.05$), n = 4.

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Study with Amino Acid Homopolymers in Liposome

The model system studies with amino acid monomers indicated that radiolytic degradation of amino acids not only occurs at side chains but also at amino and carboxyl groups. Thus, the volatile compounds produced from amino acid monomers by irradiation cannot represent the volatiles produced from proteins. To overcome this problem, a phospholipid liposome system prepared with phosphatidylcholine and phosphatidic acid was used because it better represents the cell membranes of meat (*32*). All seven amino acid polymer groups used in this study were aliphatic (poly-L-alanine, poly-L-glycine, poly-L-leucine), aliphatic hydroxyl (poly-L-threonine), basic (poly-L-histidine, poly-L-lysine), acidic (poly-L-aspartic acid, poly-L-glutamic acid), aromatic (poly-L-tyrosine), amide (poly-L-asparagine), and sulfur-containing side chain groups

October 23, 2009 | http://pubs.acs.org
Publication Date: January 2, 2004 | doi: 10.1021/bk-2004-0875.ch004

(Met-Gly-Met-Met, glutathione). A liposome containing all 7 amino acid polymer groups (used as a "reference") and 7 liposome solutions containing 6 amino acid homopolymer groups were prepared. Four 5-ml portions of samples were transferred to scintillation vials and irradiated at 0 or 5 kGy dose using a linear accelerator. Irradiation greatly influenced the amounts and profiles of volatiles in amino acid homopolymer-in-liposome systems. The volatile profiles of all 6 liposome systems that contain sulfur amino acid group were similar to that of the "reference" containing all 7 amino acid homopolymer groups (Table IX). Many new volatiles including sulfur dioxide, dimethyl sulfide, 2-methyl-2-propanol, 2-methyl-propanal, methylthio ethane, benzene, methyl ethyl disulfide, and dimethyl trisulfide were generated in "reference", and the amounts of carbon sulfide, dimethyl disulfide, and ethyl benzene increased greatly after irradiation. No sulfur volatiles, however, were produced from AA lipisome systems that did not contain sulfur containing compounds after irradiation (Table IX). The majority of newly generated and increased volatiles by irradiation were sulfur compounds indicating that sulfur-containing amino acids are among the most susceptible amino acid groups to irradiation.

Sensory panelists described the odor of irradiated amino acid homopolymers with sulfur AA as "hard-boiled egg," "boiled sweet corn," "sweet and sulfury," or "steamed vegetable," typical odor characteristics of sulfur volatile-containing samples, indicating that sulfur volatiles played the major role in the odor of the irradiated meat sample. Although nonirradiated samples also produced some sulfury notes, irradiated samples produced much stronger and astringent sulfury odor than nonirradiated ones. All liposome groups containing the "sulfur amino acids" group produced similar odor characteristics, indicating that sulfur amino acids are mainly responsible for irradiation odor as suggested by Ahn (*26*).

In summary, the production of many new volatiles from amino acids by irradiation indicated that more than one site if the amino acid side chain was susceptible to free radical attack, and many volatiles can apparently be produced by secondary chemical reactions after the primary radiolytic degradation of the side chains. Only sulfur-containing volatiles, however, produced a strong off-odor that was similar to the odor that was found as a result of the irradiation treatment of meat. The perception of odor from samples containing sulfur volatiles changed somewhat depending on the composition of other volatiles in the sample. Although some volatiles produced from non-sulfur amino acid homopolymers can interact with sulfur compounds, their roles in the odor characteristics of irradiated liposomes can be considered minor.

C. Color Changes in Meat by irradiation

Along with off-odor, another critical quality issue in irradiating meat is color change. The color changes in irradiated meat differ significantly

Table IX. Volatiles and Odor Characteristics of an Amino Acid Homopolymer Mixture containing all Amino Acid Groups or without Sulfur AA after Irradiation*

	With all AA groups		*without sulfur AA*	
	0 kGy	*5 kGy*	*0 kGy*	*5 kGy*
Volatiles	------------ Total ion counts x 10^4 ------------			
Sulfur dioxide	0^b	1210^a	0	0
1-Butene	386^a	211^b	295^a	132^b
11,1-Dimethyl cyclopropane	0	0	0^b	65^a
Pentane	0	0	0^b	183^a
1,1'-Oxybis ethane	875^a	299^b	1484^a	362^b
2-Propanone	37459^a	357^b	39551^a	377^b
Dimethyl sulfide	0^b	223^a	0	0
Carbon disulfide	454^b	2421^a	0	0
Methyl thiirane	210^a	0^b	0	0
2-Methyl-2-propanol	0^b	324^a	0^b	297^a
1,1-Dimethylethyl hydroperoxide	517^a	0^b	455^a	0^b
2-Ethoxy butane	292^a	0^b	342^a	0^b
2-Methyl propanal	0^b	115^a	0^b	146^a
Hexane	757^a	76^a	48^b	122^a
3-Methylfuran	0	0	0^b	79^a
Butanal	261^a	107^b	0^b	156^a
2-Pentene	259^a	0^b	432^a	0^b
Methylthio ethane	0^b	54^a	0	0
4-Methyl-3-hexanol	0	0	244^a	0^b
Ethyl acetate	67^a	0^b	0	0
Benzene	0^b	5083^a	0^b	9948^a
3-Methyl-butanal	108^a	139^a	377^a	268^b
1,4-Dioxane	304^a	0^b	181^a	0^b
3,3-Dimethyl-2-butanone	107^a	0^b	88^a	0^b
Dimethyl disulfide	57^b	34490^a	0	0
Toluene	251^a	0^b	358^a	0^b
Ethyl-benzene	48^b	1228^a	57^b	892^a
1,3-Dimethyl-benzene	160^a	43^b	188^a	0^b
Methyl ethyl disulfide	0^b	66^a	0	0
Octane	0	0	0^b	79^a
Xylene	0^b	145^a	0^b	79^a
Dimethyl trisulfide	0^b	7010^a	0	0
Odor characteristics of irradiated samples				
	Hard-boiled egg, sweet and sulfury, steamed vegetable		Hospital odor, alcohol, solvent, wet dog	

[a, b] Means with no common superscript within a row of the same AA groups differ significantly ($p < 0.05$), n = 4. *Contains acidic, aliphatic, aliphatic hydroxyl, amide, aromatic, basic, and sulfur amino acid groups.

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depending on various factors such as irradiation dose, animal species, muscle type, and packaging type (*33, 34, 35, 36*). Increased redness is a problem in irradiated light meat such as poultry breast and pork loin, while brown or gray discoloration is the problem in irradiated red meat. Because consumers associate the presence of pink in uncured cooked poultry with undercooking, the elimination of a persistent pink defect in irradiated light meats is very important. Although color changes in both red and light meats are important, our discussion is focused on the color changes in irradiated light meats.

Color Changes in Raw and Cooked Meat

The surface CIE color values of aerobically and vacuum-packaged raw and cooked turkey breast meat were compared by the effects of irradiation dose and storage time (Table X). Irradiation increased redness (a*-value) of both aerobically and vacuum-packaged raw turkey breast. The color changes were not localized in any specific area but evenly distributed over the whole meat sample. The increased redness was irradiation dose-dependent and was stable during the 2-week storage periods in raw meat. The result is consistent with that of Luchsinger et al. (*35*) who reported that increased red color in irradiated pork was more intense and stable with vacuum packaging than aerobic conditions during refrigerated storage. Satterlee et al. (*33*) reported that the presence of air slightly inhibited the formation of red color in irradiated bovine metmyoglobin solutions. Grant and Patterson (*37*) also reported that irradiated meat could be discolored in the presence of oxygen. Therefore, the red color formed by irradiation was produced in mainly anoxic conditions and the pigment generated by irradiation cannot be regarded as only an oxygen-related pigment. In cooked meat, the increased redness was greater inside than on the surface, and the pink color intensity inside of the cooked meat was stronger in irradiated meat than in the nonirradiated. With aerobic packaging, irradiation did not influence the surface color of cooked turkey breast. The surface color was grayish brown regardless of irradiation, and the pink color inside of aerobically packaged cooked meat also was changed to brown or yellow regardless of irradiation at 2 weeks because of pigment oxidation.

The lightness (L*-value) of raw and cooked turkey breast was not much different regardless of packaging, irradiation, and storage time. Irradiation did not affect the yellowness (b*-value) of raw turkey breast in both packaging conditions. Regardless of irradiation, b*-values of aerobically packaged raw turkey breast increased with increased storage time. Therefore, b*-value can be used as an indicator of storage time for raw meat in aerobic conditions. Irradiation, however, decreased the surface yellowness (b*-value) of cooked turkey breast with both vacuum and aerobic packaging.

Table X. CIE Color Values of Raw and Cooked Turkey Breast with Different Packaging, Irradiation, and Storage Conditions

	Aerobic packaging			*Vacuum packaging*		
Storage	*0 kGy*	*2.5 kGy*	*5.0 kGy*	*0 kGy*	*2.5 kGy*	*5.0 kGy*
Raw meat[1]						
L*-value						
0 Week	47.70	45.85^{y}	48.78	45.78^{y}	47.33	47.28^{xy}
1 Week	48.32	50.08^{x}	48.54	49.23^{x}	49.66	49.89^{x}
2 Week	48.66	49.18^{x}	48.05	44.27^{by}	47.72^{a}	45.43^{by}
a*-value						
0 Week	3.02^{c}	4.69^{b}	6.45^{a}	2.86^{cy}	5.72^{by}	6.93^{ay}
1 Week	3.04^{b}	5.28^{a}	5.61^{a}	2.90^{cy}	5.60^{by}	6.42^{ay}
2 Week	3.49^{c}	4.96^{b}	5.85^{a}	3.73^{cx}	6.77^{bx}	8.64^{ax}
b*-value						
0 Week	6.00^{aby}	5.26^{by}	6.51^{ay}	5.33	5.04^{x}	5.43
1 Week	6.39^{y}	7.35^{x}	7.21^{xy}	4.04	4.10^{y}	4.34
2 Week	7.78^{x}	8.08^{x}	8.01^{x}	5.13	5.43^{x}	5.40
Cooked meat (internal color)[2]						
L*-value						
0 Week	83.98^{xy}	83.82	82.99	84.36^{a}	84.78^{a}	81.49^{b}
1 Week	84.91^{x}	82.66	82.84	83.50	84.01	81.39
2 Week	81.79^{y}	82.16	81.85	82.79	82.41	82.98
a*-value						
0 Week	8.09^{c}	9.16^{bx}	10.81^{ax}	7.84^{cy}	9.47^{b}	12.40^{ax}
1 Week	8.56^{b}	9.85^{ax}	10.50^{ax}	9.42^{bx}	9.79^{b}	11.55^{ax}
2 Week	8.48	7.84^{y}	8.10^{y}	7.04^{cy}	8.82^{b}	9.40^{ay}
b*-value						
0 Week	14.96^{x}	15.88^{x}	14.97^{x}	15.65^{y}	15.82^{x}	15.94^{x}
1 Week	14.58^{x}	15.49^{x}	15.39^{x}	16.65^{x}	15.86^{x}	16.11^{x}
2 Week	13.53^{y}	14.12^{y}	13.69^{y}	13.82^{z}	13.28^{y}	12.75^{y}

$^{a-c}$Different letters within a row with the same packaging are different ($P < 0.05$).

$^{x-z}$Different letters within a column of the same irradiation dose are different ($P < 0.05$).

L*-value: lightness, a*-value: redness, b*-value: yellowness. n = 8.

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Production of Carbon Monoxide

Furuta et al. (*38*) and Woods and Pikaev (*39*) reported that a considerable amount of carbon monoxide (CO) gas was produced by radiolysis of organic components in irradiated frozen meat and poultry. Nam and Ahn (*40*) attributed the increased red color in irradiated turkey meat to the formation of carbon monoxide-myoglobin (CO-Mb) complexes. The CO-Mb complex is more stable than oxymyoglobin because of the strong binding of CO to the iron-porphyrin site on the myoglobin molecule (*41*).

Table XI. The Production of CO in Raw and Cooked Turkey Breast with Different Packaging, Irradiation, and Storage Conditions[1]

	Aerobic packaging			*Vacuum packaging*		
Storage	*0 kGy*	*2.5 kGy*	*5.0 kGy*	*0 kGy*	*2.5 kGy*	*5.0 kGy*
	Unit (ppm[1])					
Raw meat[2]						
0 Week	0^{cz}	328^{bx}	593^{ax}	0^{cy}	445^{b}	999^{ax}
1 Week	45^{by}	359^{ax}	509^{ax}	19^{cx}	394^{b}	560^{ay}
2 Week	74^{x}	134^{y}	144^{y}	6^{cxy}	365^{b}	533^{ay}
Cooked meat[3]						
0 Week	220^{cx}	319^{bx}	456^{ax}	227^{cx}	370^{bx}	575^{ax}
1 Week	230^{bx}	210^{by}	261^{ay}	154^{cy}	336^{bxy}	558^{ax}
2 Week	134^{y}	181^{y}	227^{y}	130^{cy}	289^{by}	450^{ay}

[1] Concentration in headspace (14 ml) from 10 g meat. [a-c] Different letters within a row with same packaging are different ($P < 0.05$). [x-z] Different letters within a column with same irradiation dose are different ($P < 0.05$).

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To identify gaseous compounds that can be the sixth ligand of heme pigments in irradiated raw and cooked turkey breast, the amount of CO was analyzed using a GC and FID detectors with a Nickel catalyst. The production of CO in irradiated turkey breast was irradiation-dose dependent (Table XI). CO has a strong affinity to heme pigments and can be considered as a possible sixth ligand of myoglobin, which could be responsible for the red or pink color in irradiated turkey breast. Watts et al. (*42*) found that fresh meat exposed to low levels of CO gas turned red with the formation of CO myoglobin. Irradiation generated CO gas in both aerobically and vacuum-packaged meat, but the vacuum-packaged turkey breast showed higher CO levels than those of the aerobically packaged turkey breast. After 2 weeks of storage, the amount of CO decreased in aerobically packaged irradiated turkey breast. Most CO gas produced by irradiation escaped under aerobic conditions. On the other hand, a considerable amount of CO remained in vacuum-packaged irradiated turkey breast, and it can be considered that the gas was related to the vivid red color that existed in the vacuum-packaged meat samples stored for 2 weeks. Irradiation, as well as cooking, produced carbon monoxide. CO was also detected in nonirradiated meat samples, but it increased proportionally to the irradiation dose.

Oxidation-Reduction Potential

The oxidation-reduction potential (ORP) of raw and cooked turkey breast meat initially was decreased by irradiation in both aerobically and vacuum-packaged conditions, but vacuum-packaged meat had much lower ORP values than the aerobically packaged meat's (Table XII). Irradiation can provide meat with a strongly reduced environment. Swallow (*43*) reported that hydrated electrons, one of the radicals produced by irradiation, could act as a very powerful reducing agent, and reacted with ferricytochrome to produce ferrocytochrome. Cornforth et al. (*44*) reported that hemochrome formation was promoted by reducing conditions and prevented by oxidizing conditions. We postulate that the iron of myoglobin was changed to a ferrous iron under the reduced conditions of irradiated turkey breast, and the reduced iron had stronger affinity to accept a ligand and produced a red color. In irradiated raw and cooked turkey breast, therefore, the ORP explains the higher a*-values in vacuum-packaged meat samples than in aerobically packaged meat.

As the storage time increased, however, the ORP values in irradiated raw turkey breast increased, whereas the ORP values in nonirradiated turkey breast decreased in both packaging conditions. Generally, the ORP values of raw meats declines during the initial storage due to the oxygen consumption in meat tissues or by microorganisms. Cornforth et al. (*44*) reported that microbial growth decreased ORP values and thus increased reducing capacity. After 2 weeks of storage, the differences of ORP between nonirradiated and irradiated raw turkey breasts disappeared or reverted within the same packaging condition. Although the ORP values decreased during irradiation, this reduced condition produced in irradiated raw meat was not maintained during storage. The result did not coincide with the red color of stored irradiated raw meat, because the color of irradiated raw meats was still redder or pinker than nonirradiated meats during storage. The red pigments generated by irradiation were fairly stable against the increased oxidative environmental stress during the storage time. We

Table XII. Oxidation-Reduction Potential (ORP)* of Raw and Cooked Turkey Breast Meat with Different Packaging, Irradiation Dose, and Storage Conditions

	Aerobic packaging			*Vacuum packaging*		
Storage	*0 kGy*	*2.5 kGy*	*5.0 kGy*	*0 kGy*	*2.5 kGy*	*5.0 kGy*
	Unit (mV)					
Raw meat[1]						
0 Week	-15.7^{ax}	-174.7^{bz}	-91.2^{bz}	-74.0^{ax}	-193.2^{b}	-279.0^{cy}
1 Week	-19.0^{cx}	11.7^{by}	34.5^{ay}	-147.7^{bz}	-127.2^{ab}	-109.7^{ax}
2 Week	-58.7^{by}	46.2^{ax}	65.5^{ax}	-113.5^{y}	-145.2^{a}	-134.7^{x}
Cooked meat[2]						
0 Week	-19^{ay}	-49^{by}	-62^{by}	-48^{ay}	-71^{ay}	-104^{by}
1 Week	102^{ax}	68^{bx}	75^{bx}	-49^{ay}	-53^{bxy}	-50^{abx}
2 Week	113^{ax}	84^{bx}	82^{bx}	-18^{ax}	-41^{abx}	-59^{bx}

$^{a-c}$Different letters within a row with the same packaging are different ($P < 0.05$),
$^{x-z}$Different letters within a column of the same irradiation dose are different ($P < 0.05$).
*ORP indicates the degree of reducing potential. n = 8.
SOURCE: [1]Reproduced with permission from reference 40. Copyright 2002 Elsevier. [2]Reproduced with permission from reference 47. Copyright 2002 J. Food Sci.

can expect that ligand molecules having high affinity to heme pigment could be generated by irradiation.

In cooked meat, both undenatured and denatured heme pigments may have been involved in heme-complex formations (with ligands available under the experimental conditions), which is important for the pink color formation in cooked turkey. The decreased ORP by irradiation in aerobically packaged cooked meat, however, was not low enough to produce the distinct pink color. The ORP increased faster under aerobic than under vacuum conditions during storage. Within each packaging condition, however, irradiated samples had lower ORP than the nonirradiated samples during storage. Vacuum packaging maintained the decreased ORP conditions produced by irradiation during 14 days of storage. The color of irradiated meat was still pinker than nonirradiated ones even after 14 days of storage under vacuum. The surface pink color generated by irradiation was stable during the storage with vacuum packaging. This indicated that some compounds that can become the sixth ligand of heme pigments were generated by irradiation.

Reflectance and Absorption Spectra of Meat and Drip

At 1 week of storage, absorption spectra of meat drips from aerobically packaged turkey breast were characterized by the absorption maxima of 536 and 566 nm (Figure 1). Compared with the spectra of nonirradiated samples, irradiation moved two absorption peaks that existed in the 500- to 600-nm region into shorter wavelengths. The changes in absorption maxima indicated that the color pigments of irradiated meat were not oxymyoglobin (absorption maxima at 543 nm and 580 nm) or nitric oxide-myoglobin (absorption maxima at 547 nm and 578 nm), because the two absorption maxima of irradiated meat were shorter than the usual absorption maxima of oxy- or nitric oxide-myoglobin (Figure 2). Peak intensity could not be compared due to the different concentrations of samples obtained from meat juices.

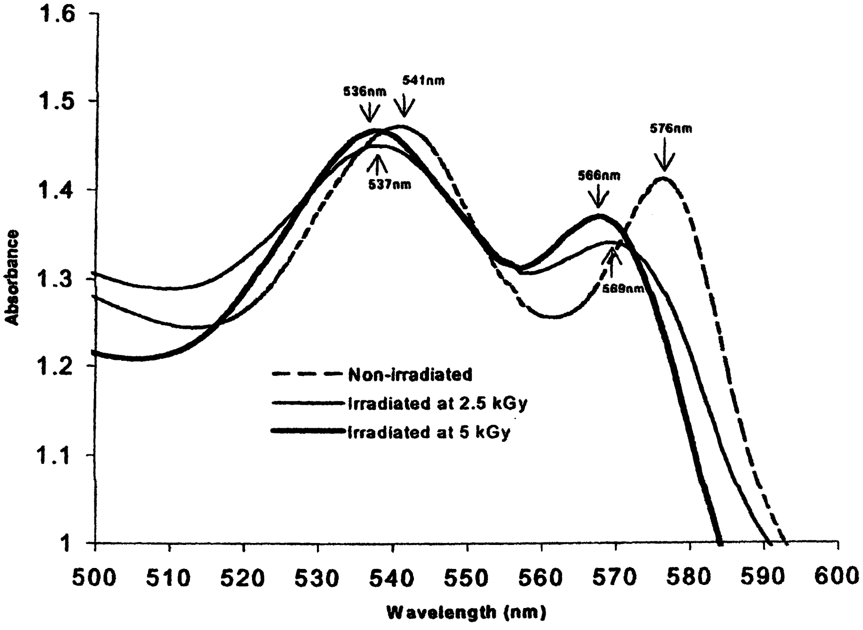


Figure 1. Absorption spectra of meat juice from aerobically packaged turkey breast with different irradiation doses. (Reproduced with permission from reference 40. Copyright 2002 Elsevier)

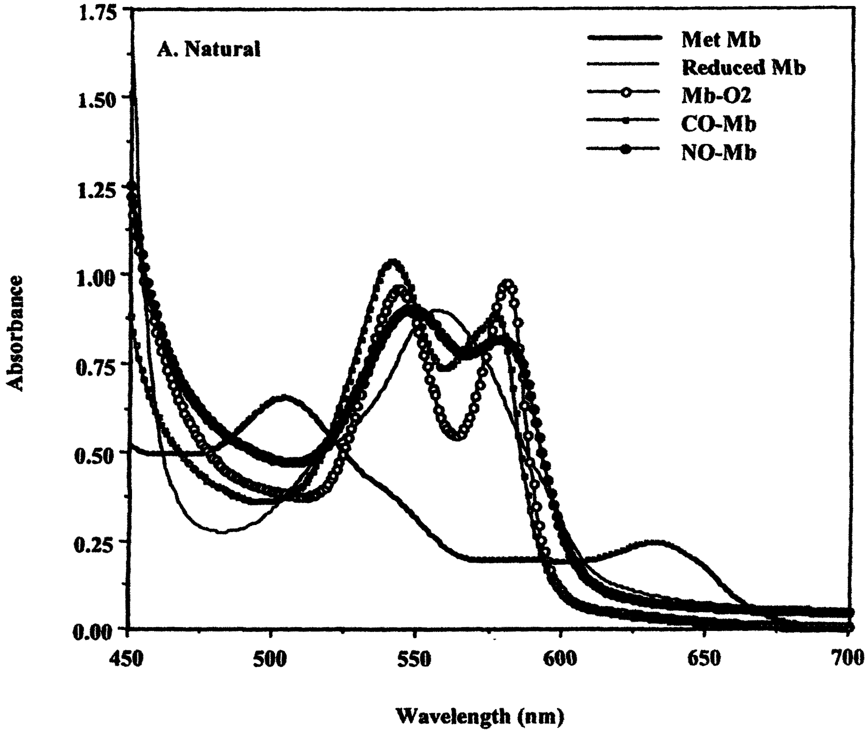
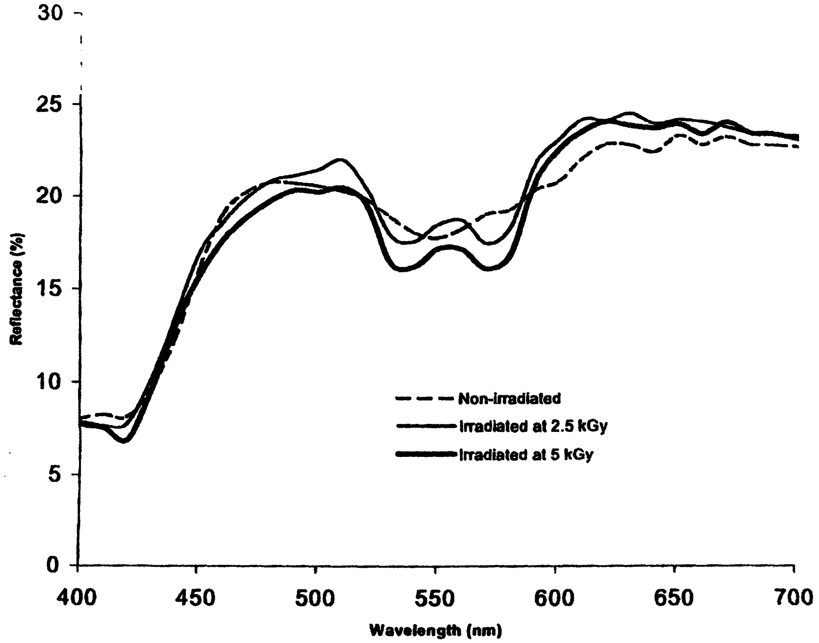


Figure 2. Absorption spectra of various myoglobin (Mb) forms in solution. Carbon monoxide-heme pigment complexes are responsible for the pink color in irradiated raw turkey breast meat. (Reproduced with permission from reference 40. Copyright 2002 Elsevier)

Reflectance spectra of aerobically packaged turkey breast also confirmed the result from the absorption spectra (Figure 3). The reflectance spectra from nonirradiated meat surfaces showed that the color pigments consisted of mainly deoxymyoglobin. Two reflectance minima were formed by irradiation in the range of 500- to 600-nm, and the intensity of the minima from 5 kGy-irradiated samples was lower than that of 2.5 kGy-irradiated samples. The wavelengths of reflectance minima in 5 kGy-irradiated meat samples were not different from those in 2.5 kGy-irradiated samples. In the range between 600- and 700-nm (red color spectrum), irradiated samples had higher reflectance values than nonirradiated samples.



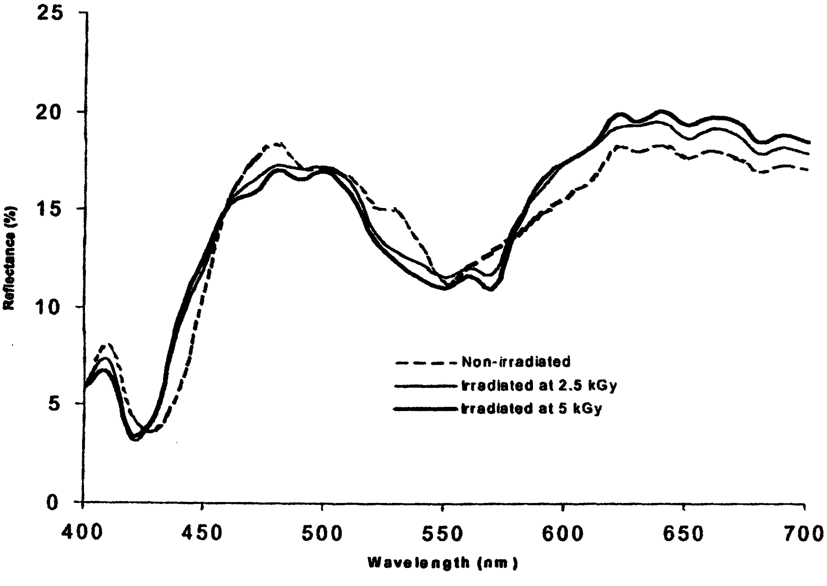


Figure 3. Reflectance spectra of aerobically (Top) or vacuum-packaged (bottom) turkey breast with different irradiation doses. (Reproduced with permission from reference 40. Copyright 2002 Elsevier)

Overall, the reflectance of vacuum-packaged turkey breast was lower than that of the aerobically packaged turkey breast (Figure 3). The spectra of vacuum-packaged, nonirradiated turkey breast showed that a large proportion of the pigments were reduced-myoglobin or hemoglobin. As in aerobically packaged meat, irradiation formed two distinctive reflectance minima in the range of 500- to 600-nm, and the reflectance minima were sharper and lower than those of aerobically packaged meat. As a result, the meat had a more intense red color in irradiated vacuum-packaged samples than in aerobically packaged samples. In the range between 600- and 700-nm (red color spectrum), irradiated samples showed higher reflectance values than the nonirradiated samples in aerobically packaged meat.

Correlations

The correlation coefficients between CIE color values, irradiation dose, storage time, and the other analytical values are shown in Table XIII. In both aerobically and vacuum-packaged turkey breast, the a*-values of turkey breast were positively correlated with the irradiation dose and the amount of CO gas produced ($P<0.01$). The increased a*-values in irradiated turkey breast were maintained regardless of increased ORP and lipid oxidation during the 2 weeks of storage. The result shows that the initial red or pink pigments formed by irradiation were stable against oxidation during the storage time. In aerobically packaged meat, b*-value was positively correlated with L*-value, TBARS value, and storage time. Therefore, b*-value can be a reliable indicator of storage history or lipid oxidation in aerobically packaged meat. Table XIV shows Pearson correlation coefficients between CIE color values and other factors in irradiated cooked turkey breast. In vacuum-packaged cooked turkey breast, the a*-values of both surface and inside were positively correlated with the irradiation dose and the amount of CO gas produced. Although significant correlation between a*-value and ORP was found in only inside meat color, the increased a*-values by irradiation were highly correlated with ORP of meat surface at week zero ($r = -0.73$). Therefore, the increased a*-values of irradiated cooked meat with vacuum packaging could be attributed to the decreased ORP and the formation of heme pigment-CO complex. The result also showed that the pink pigment formed by irradiation was stable against the oxidation during the storage. In aerobically packaged cooked turkey breast, the a*-value of the meat surface was not affected by irradiation because of oxidation.

In summary, the mechanism of color conversion of raw and cooked turkey breast meat by ionizing radiation can be explained as follows: irradiation generated a few gaseous compounds, one of which was CO, and provided more reduced environments to the heme pigments, which increased the CO-heme pigments complex formation and the intensity of pink color. CO-heme pigment was the major color component responsible for the pink color in irradiated raw and cooked turkey breast, and the pigment formed was stable under vacuum packaging.

Table XIII. Pearson Correlation Coefficients between Color Values, Irradiation Dose, Storage Time, CO, Redox Potential, and TBARS of Raw Turkey Breast

	a-value*	*b*-value*	*IR*[a]	*Storage*	*CO*	*ORP*[b]	*TBARS*[c]
Aerobic packaging							
L*-value	0.22	0.69*	0.08	0.44	0.12	0.61	0.18
a*-value	.	0.28	0.93**	0.01	0.80**	0.12	0.38
b*-value		.	0.23	0.90**	-0.12	0.75*	0.74*
IR			.	0.00	0.74*	0.21	0.43
Storage				.	-0.38	0.63	0.78*
CO					.	-0.23	-0.17
ORP						.	0.65
Vacuum packaging							
L*-value	0.05	-0.76*	0.20	-0.21	0.23	-0.09	-0.30
a*-value	.	0.33	0.88**	0.26	0.79**	-0.39	0.58
b*-value		.	0.17	0.03	0.23	-0.23	0.73**
IR			.	0.00	0.89**	-0.44	0.47
Storage				.	-0.23	0.37	-0.19
CO					.	-0.74*	0.63
ORP						.	-0.42

[a] Irradiation dose, [b] Oxidation-reduction potential,
[c] 2-thiobarbituric acid reactive substances.

*Value with significant correlation ($P < 0.05$). n = 18.

**Value with significant correlation ($P < 0.01$)

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Table XIV. Pearson Correlation Coefficients between Color Values and other Factors in Cooked Turkey Breast Meat

	Aerobic packaging			*Vacuum packaging*		
	L-value*	*a*-value*	*b*-value*	*L*-value*	*a*-value*	*b*-value*
Surface color						
Irradiation dose	-0.12	0.46	-0.38	-0.32	0.88**	-0.87**
Storage time	-0.03	-0.83**	0.51	-0.32	0.05	0.12
ORP[b]	0.12	-0.88**	0.38	-0.41	-0.55	0.45
TBARS value	0.08	-0.70*	0.31	0.52	-0.77*	0.25
Carbon monoxide	0.06	0.85**	-0.60	0.21	0.76*	-0.73*
Internal color						
Irradiation dose	-0.19	0.40	0.12	0.05	0.80*	0.05
Storage time	-0.64*	-0.34	-0.76*	0.25	-0.40	-0.80*
ORP	-0.55	-0.33	-0.55	0.26	-0.79*	-0.39
TBARS value	-0.53	-0.36	-0.50	0.33	0.12	0.71*
Carbon monoxide	0.07	0.69*	0.52	-0.55	0.88**	0.11

[a]n = 72, [b]Oxidation reduction potential. *Value with significant correlation ($P < 0.05$). **Value with significant correlation ($P < 0.01$).

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D. Remedies to Off-Odor Production and Color Changes

Double-Packaging

Packaging turned out to be a major factor influencing the amounts and types of volatiles detected in irradiated meat. Exposing irradiated meats to aerobic conditions increases ORP and $CO:O_2$ competition, which decreases pink color intensity. Sulfur compounds, the most critical volatiles for off-odor development in irradiated meat, can easily be eliminated under aerobic conditions. To solve off-odor problems in irradiated meat, one approach is to use a double-packaging concept. In the double-packaging method, meat is individually packaged in an oxygen permeable zipper bag, where a few of these bags are then placed in a larger oxygen impermeable vacuum bag.

Sliced raw turkey breast and thigh meats were aerobically, vacuum- or double (vacuum/aerobic)-packaged, electron beam-irradiated at 2.5 kGy, and then stored under a refrigerated temperature (*45, 46*). For the double-packaged samples, the outer vacuum bags were removed after 5, 7, or 9 d of refrigerated storage. The results showed that irradiation and aerobic packaging promoted the production of aldehydes (propanal and hexanal) related to lipid oxidation in turkey breast and thigh meats. Vacuum-packaged irradiated samples retained S-volatile compounds (methanethiol, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide), mainly responsible for the irradiation off-odor, during the storage. Exposure of double-packaged irradiated turkey meats to aerobic conditions by removing the outer vacuum bags a few days before the test was effective in controlling both lipid oxidation-dependent (aldehydes) and radiolytic off-odor (S-compounds) volatiles. The a*-values of raw turkey breast and thigh meats increased by irradiation regardless of packaging conditions. The a*-value of double-packaged meats was lower than that of the vacuum-packaged meats, but it was not significant. Thus, the use of double-packaging alone was not enough to reduce the pink color of irradiated raw turkey meat. When both lipid oxidation and irradiation off-odor should be minimized without any additional additives, use of double packaging may be applied since it is an excellent method for turkey meats.

Double Packaging and Additive Combinations

The decrease of ORP in turkey breast by irradiation (*40, 47*) suggests that irradiation is a source of solvated electrons. Rapp et al. (*48*) reported that solvated electrons attack the distal histidine of methemoglobin, which drives out the ligand at the sixth site to allow hemochrome formation via a covalent bond of the distal histidine to the iron atom. This process is accelerated when a substantial amount of hydroxide anion is present. Lowering pH will decrease the amount of hydroxide anion present and therefore decrease redness. Kieffer et al. (*49*) reported that incorporation of citric acid (0.3%) in cooked turkey reduced the redness by 63% compared to the control samples. Citric acid also reduced the pink color in nitrite treated samples. Effects of double-packaging and acid (citric or ascorbic acid) combinations on color, lipid oxidation and volatiles of irradiated raw turkey breast, however, showed that acid did not affect the a*-values but increased the L*-values of meat after irradiation (*50*). Citric acid promoted lipid oxidation of irradiated turkey meat, whereas ascorbic acid had an antioxidant effect.

Antioxidants such as free radical terminators or metal chelating agents are commonly used in meat to reduce lipid oxidation and to improve sensory quality of meat (*51, 52*). Huber et al. (*53*) found that the use of antioxidants such as ascorbate, citrate, tocopherol, gallic esters, and polyphenols was effective in reducing the off-odor of irradiated meat. Graf (*54*) reported that ferulic acid has an UV-absorption capability and can reduce radiation-induced oxidative reactions. The incorporation of antioxidants into cell membranes via dietary treatments has been shown to stabilize lipids in membranes and reduce the extent of lipid oxidation in meat during storage (*55, 56, 57*). Patterson and Stevenson (*20*) reported that irradiated meat from chickens, reared on diets supplemented with both α-tocopherol and ascorbic acid, produced qualitatively similar volatile component patterns, but the yield of volatile compounds was substantially reduced compared to control. The antioxidant effects of dietary tocopherol in chicken meat, however, differ among muscle types (*14*).

Our studies with double packaging and antioxidant combinations indicated that sesamol+α-tocopherol (S+E) and gallate+α-tocopherol (G+E) combinations were very effective in preventing lipid oxidation during storage, and the TBARS values of the antioxidant-treated meats were lower than nonirradiated vacuum-packaged raw meat at 10 d (Table XV). The antioxidant effect on lipid oxidation of turkey meat was even more distinct after cooking. The TBARS values of irradiated turkey meat increased rapidly after cooking, but those with antioxidants did not. Therefore, the problem of lipid oxidation in aerobically or double-packaged irradiated raw and cooked turkey breast could be solved by adding combined antioxidants.

Table XV. TBARS and Color a* Values of Raw Turkey Breast with Different Packaging and Antioxidants

	NonIr	*Irradiated*				
	Vacuum	*Vacuum*	*Aerobic*	*Double pkg*[1]		
	Pkg	*pkg*	*pkg*	*None*	*S+E*[2]	*G+E*[3]
TBARS	----------------------	----------------------	(mg MDA/kg meat)	----------------------	----------------------	----------------------
0 d raw	0.66^{by}	0.84^{ay}	0.91^{ay}	0.83^{ay}	0.42^{dy}	0.55^{c}
10 d raw	0.72^{cy}	0.84^{cy}	2.18^{ax}	1.61^{by}	0.53^{cx}	0.53^{c}
Cooked	1.12^{dx}	1.67^{cx}	2.37^{ax}	2.09^{bx}	0.54^{ex}	0.64^{e}
Color a* values						
0 d raw	4.42^{cz}	7.95^{ay}	7.15^{bx}	7.74^{axy}	6.95^{by}	6.74^{bx}
10 d raw	4.67^{dz}	7.89^{ay}	5.66^{cy}	6.98^{by}	4.68^{dz}	5.63^{cy}
Cooked	7.50^{cx}	10.04^{ax}	5.58^{dy}	8.62^{bx}	7.51^{cx}	5.75^{dy}

[1] Vacuum packaged for 7 d then aerobically packaged for 3 d, [2] Sesamol (100 ppm) and α-tocopherol (100 ppm) added, [3] Gallic acid (100 ppm) and α-tocopherol (100 ppm) added.
$^{a-e}$Different letters within a row are significantly different ($P < 0.05$), n = 4.
^{x,y}Different letters within a column with same parameter are significantly different ($P < 0.05$). SOURCE: Reproduced with permission from reference 46. Copyright 2003 J. Food Sci.

Antioxidants lowered the L*-value of vacuum-packaged irradiated meat by about 2 units and a* value by 1 unit (Table XV). The a*-value of aerobically packaged irradiated meat was lower than that of vacuum-packaged meat, but was still higher than nonirradiated control. After 10 day of refrigerated storage, the redness of double-packaged meat decreased significantly. Furthermore, the combination of antioxidants with double packaging showed a synergistic effect in reducing the redness of irradiated meat: the presence of oxygen should have accelerated the dissociation of CO-Mb, while antioxidants should have inhibited the radiolytic generation of CO.

Irradiated cooked turkey breast meat from double packaging and antioxidant combinations also produced significantly lower a* values than the vacuum-packaged irradiated cooked meat. Gallate+α-tocopherol (G+E) was significantly more effective in reducing the redness than S+E. Therefore, G+E in combination with double packaging can be effective in controlling off-color in irradiated raw and cooked turkey breast meat.

Little difference in volatile profiles between vacuum-packaged irradiated and doubly packaged irradiated meats at day zero was found, because both samples were under vacuum conditions during irradiation (Table XVI). Antioxidant treatments lowered total volatiles in raw turkey meat, and propanal was not detected when antioxidants were added. After 10 d of refrigerated storage, volatile profiles of irradiated turkey breast were highly dependent upon antioxidant and packaging conditions. Sulfur volatiles were not detected in irradiated aerobically or double-packaged meat. A 3-day exposure to aerobic conditions was enough for the sulfur volatiles to escape from the meat. However, aerobically packaged irradiated meat without antioxidants produced large amounts of aldehydes (propanal, hexanal) and 2-butanone at 10 d, which coincided with the degree of lipid oxidation (measured by TBARS). Double-packaged meat had few lipid oxidation products as compared with aerobically packaged meat, but the addition of antioxidants significantly reduced the amount of pentane. Therefore, the combination of double packaging (vacuum for 7 d then aerobic for 3 d, V7/A3) with antioxidants in irradiated raw turkey breast was very effective in reducing total sulfur volatiles responsible for the irradiation off-odor without any problem in lipid oxidation.

The beneficial effects of double packaging and antioxidant combinations on volatiles were clearly shown in irradiated cooked turkey breast (Table XVI). Double packaging was more effective than vacuum packaging in reducing sulfur volatiles and lipid oxidation-dependent volatiles, as compared with aerobic packaging. However, the combination of antioxidant with double packaging was more effective in reducing both sulfur and lipid oxidation volatiles in irradiated cooked meat. The total amounts of sulfur volatiles in double-packaged irradiated turkey meat with antioxidants were only about 5-7% of the irradiated vacuum-packaged cooked meat without antioxidants. Production of most aldehydes in irradiated cooked turkey breast was prevented by using antioxidants and double packaging.

Table XVI. Sulfur Compounds and Aldehydes of Raw and Cooked Turkey Breast with Different Packaging and Antioxidants

	NonIr	*Irradiated*				
Sulfur compounds	*Vacuum pkg*	*Vacuum pkg*	*Aerobic pkg*	*Double pkg*[1] *None*	*S+E*[2]	*G+E*[3]
	(Total ion counts × 10^4)					
Raw meat						
Dimethyl sulfide	1,304^{b}	1,990^{a}	140^{d}	831^{c}	676^{c}	546^{c}
Carbon disulfide	258^{b}	306^{a}	0^{c}	0^{c}	0^{c}	0^{c}
Dimethyl disulfide	0^{b}	22,702^{a}	0^{b}	32^{b}	0^{b}	43^{b}
Dimethyl trisulfide	0^{b}	554^{a}	0^{b}	0^{b}	0^{b}	0^{b}
Cooked meat						
Dimethyl sulfide	1,008^{b}	2,032^{a}	451^{d}	1,005^{b}	689^{c}	588^{cd}
Carbon disulfide	419^{a}	339^{ab}	210^{b}	271^{ab}	278^{ab}	374^{a}
Dimethyl disulfide	0^{b}	17,861^{a}	342^{b}	940^{b}	412^{b}	210^{b}
Dimethyl trisulfide	0^{b}	1,007^{a}	0^{b}	118^{b}	0^{b}	0^{b}
Propanal	233^{d}	2,272^{c}	8,637^{a}	5,962^{b}	38^{d}	427^{d}
Butanal	0^{e}	127^{d}	592^{a}	195^{c}	302^{b}	226^{c}
Pentanal	62^{c}	875^{c}	3,014^{a}	1,667^{b}	0^{c}	31^{c}
Hexanal	0^{b}	3,734^{b}	37,617^{a}	9,686^{b}	0^{b}	0^{b}
3-Methyl butanal	0^{c}	100^{b}	223^{a}	204^{a}	131^{b}	142^{b}

[1]Vacuum packaged for 7 d then aerobically packaged for 3 d, [2]Sesamol (100 ppm) and α-tocopherol (100 ppm) added, [3]Gallic acid (100 ppm) and α-tocopherol (100 ppm) added. [a-e]Different letters within a row of same meat are significantly different ($P < 0.05$). n = 4. SOURCE: Reproduced with permission from reference 46. Copyright 2003 J. Food Sci.

In summary, antioxidants significantly reduced lipid oxidation and volatile aldehydes. Packaging was the most critical factor in the development of irradiation off-odor in meat. The combination of antioxidants and double packaging (V7/A3) was effective in controlling the oxidative quality changes of irradiated raw and cooked meat. Among the antioxidant and double packaging treatments, both S+E and G+E with double packaging were effective in reducing pink color, off-odor, and lipid oxidation of irradiated raw and cooked turkey breast, but G+E with double packaging was the most effective in reducing the pink color in cooked turkey breast meat.

Conclusion

Irradiation accelerates lipid oxidation, changes color, and produces off-odor in meat. However, lipid oxidation in irradiated meat becomes a problem only when meat is irradiated and stored under aerobic conditions. Irradiation increases the redness of light meat but turns the red meat to a brown color. The mechanisms involved in the color changes in light and dark meats by irradiation are different. The pink color in irradiated light meat is characterized as a carbon monoxide-heme pigment complex. Irradiation produces carbon monoxide from meat components and the production of carbon monoxide in meat is dependent on irradiation dose. Irradiation also increases the reducing power of meat, which facilitates formation of carbon monoxide-myoglobin complex, enhancing the red color intensity of heme pigments. Irradiation-induced off-odor in meat results from sulfur-containing compounds produced by radiolytic degradations of methionine and cystene; while lipid oxidation products have a minor effect on the off-odor of irradiated meat. The sulfur compounds could be easily removed by storing the irradiated meat under aerobic conditions, or by using double packaging that was found to be effective in eliminating irradiation off-odor. Combinations of antioxidants (S+T or G+T) with double packaging were observed to be greatly effective in preventing oxidative changes and off-odor, and reduced color changes in raw and cooked turkey breast meat. Most of the irradiation studies were done with raw meat because irradiation has not been approved for processed or precooked ready-to-eat meat products. Therefore, future studies should be focused on flavor, color and taste changes in processed and precooked ready-to-eat meat products by irradiation. Methods that prevent quality changes in the irradiated, and processed or precooked ready-to-eat meat products should also be developed.

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Chapter 5

Irradiation of Ready-to-Eat Meats: Eliminating *Listeria monocytogenes* While Maintaining Product Quality

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Listeria monocytogenes, a food-borne pathogen, is a common contaminant on ready-to-eat (RTE) meat products such as frankfurters, bologna, ham and deli turkey meat. A number of food-borne illness outbreaks have been attributed to this microorganism. Since 1998, over 90 million pounds of RTE meats have been recalled due to contamination with *L. monocytogenes*. Ionizing radiation can eliminate *L. monocytogenes* from RTE meat products. The radiation resistance of *L. monocytogenes* is dependent on the RTE meat formulation and the genetic characteristics of the contaminating strain. Ionizing radiation can also impact product quality factors including color, lipid oxidation, and generation of volatile sulfur compounds and hydrocarbons. As with elimination of microorganisms, effects of ionizing radiation on product quality are also product specific.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Incidence and Radiation Resistance

Listeria monocytogenes causes an estimated 2,500 cases of food-borne illness, and 500 deaths, annually in the United States (*1*). The mortality rate due to listeriosis, among susceptible populations, is approximately 20% (*1*). Many of these illnesses have been associated with consumption of contaminated ready-to-eat meat products such as frankfurters and deli meats (*2-6*). *L. monocytogenes* is capable of growth at refrigerated temperatures and in high salt environments, and such growth produces no apparent signs of spoilage in food products (*7*). Ready-to-eat (RTE) meat products are cooked as a processing step, with contamination occurring between the cooking and packaging steps. Because *L. monocytogenes* is capable of growth at low temperatures, post-process contamination with a relatively small number of microorganisms ($<10^2$ CFU/g) could result in a microbial load of $>10^6$ CFU/g at the end of a 4 week refrigerated storage period (*8-10*). Due to the high mortality rate associated with listeriosis the USDA's Food Safety Inspection Service (FSIS) has instituted a zero tolerance policy for *L. monocytogenes* in RTE meat products (*11*).

Post-process contamination of RTE meat products with *L. monocytogenes* is well documented. In 1998 approximately 2.5% of ready-to-eat meat products tested by the USDA's FSIS were positive for *L. monocytogenes* (*12*). In a recent survey of frankfurters obtained from several commercial plants, approximately 1.6% tested positive for *L. monocytogenes* (*13*). In a review of microbiological testing programs for the years 1990 to 1999, approximately 1.31% of small diameter sausages (frankfurters) and 5.16% of ham and sliced luncheon meats tested positive for the presence *of L. monocytogenes* (*14*).

Table I. Large Recalls (Over 100, 000 lbs) of RTE Meat Products Due to Contamination with *Listeria monocytogenes* (*5*)

Year	*Case No.*	*Product*	*Pounds Recalled*
2002	090-2002	RTE Turkey, Various	28,000,000
2002	098-2002	RTE Turkey, Various	4,200,000
2000	076-2000	RTE Poultry, Various	16,895,000
2000	065-2000	Weiners	900,000
1999	046-99	Beef Frankfurters	2,100,000
1999	035-99	Bacon Chips	126,739
1999	005-99	RTE Meats, Various	35,000,000
1998	044-98	Hot Dogs/Packaged Meats	35,000,000
1998	035-98	Frankfurters	1,734,002

A risk assessment completed by the U. S. Food and Drug Administration (FDA), the USDA's FSIS, and the Centers for Disease Control and Prevention found that 7.6% of frankfurters tested positive for *L. monocytogenes* (2). In that same study, non-reheated frankfurters represented the top risk for listeriosis among the 20 product categories evaluated on a per serving basis. Since 1998 over 90 million pounds of RTE meat products have been recalled due to contamination with *L. monocytogenes* (*5*). A listing of the larger recalls of RTE meats is shown in Table I. Products including bacon bits, beef jerky, roast beef, frankfurters (hot dogs), ham, and turkey have tested positive for *L. monocytogenes* and have been recalled as a result (*5*).

Of the 14 serotypes currently identified for *L. monocytogenes* serotypes 1/2a, 1/2b, and 4b account for 95% of illness in humans, with serotype 4b being responsible for most illnesses in North America (*15*). In a comprehensive survey on the recovery of *L. monocytogenes* from commercial hot dog packs, Wallace et al. (*13*) found that approximately 90% of the isolates were 1/2a while the remainder were primarily serotype 4b. It should be noted that the high percentage of positive packs (16%), primarily serotype 1/2a, from Plant 133 (Table II) raised the overall positive rate in that survey (*13*). While some hot dog packs were found to contain *L. monocytogenes* strains of more than one serotype, the vast majority of packs contained a homogeneous population (*13*). *L. monocytogenes* detection rates, and serotype information are presented in Table II.

Table II. *L. monocytogenes* Recovery Rates and Strain Characteristics

Facility Code	*Frankfurter Type*	*% Packs Positive*	*Predominant Ribotype (%)**	*Serotype*
94	Turkey	0.07	A (100)	1/2a
133	Turkey	16.0	A (100)	1/2a
172	Beef	0.11	A (100)	1/2a
344	Beef, Pork & Chicken	0.16	F (30)	4b
			B (30)	4b
			O (20)	NT
			N (10)	4b
367	Pork	1.5	A (82)	1/2a
385	Pork and Beef	0.08	G (100)	NT
439	Pork and Beef	2.2	A (100)	1/2a

* Predominant ribotype was assigned a letter code based on similarity between isolates for the basis for the study (*13*). Facility codes were generated randomly for anonymity (*13*).

RTE Meat Formulation. There is relatively little data available pertaining to elimination of *L. monocytogenes* from RTE meat products using ionizing radiation prior to 1999. Frankfurters and bologna are fine emulsion sausages that can vary greatly in formulation (*13, 16*). Meats and meat mixtures used in frankfurters and bologna can include (but are not limited to) beef, pork, chicken, and turkey. Additives can include sodium nitrite, sodium chloride, phosphates, erythorbate, ascorbate, etc. Extenders and binders, used to increase product firmness and to reduce purge (fluid loss), can include products such as lactose free whey, soy protein concentrate, various flours, carrageenan, yeast lysate, etc. Sweeteners can include anything from glucose to high fructose corn syrup. Antimicrobial compounds including organic acids, sodium or potassium lactate, and sodium diacetate can be added to the product emulsion or applied to the product surface to inhibit the growth of *L. monocytogenes*. In short, there is no "standard" frankfurter or bologna formulation, but rather a complex family of widely differing formulations within a class of products. Other types of RTE meats exhibit the same variability in product formulation.

L. monocytogenes **D_{10} value.** Recent studies have elucidated the phenomenon of variability in *L. monocytogenes* radiation resistances when inoculated onto different RTE meat products. The radiation resistance of food-borne pathogens is typically expressed as either a D_{10} value, the ionizing radiation dose required to eliminate one $\log_{10}$ of the pathogen, or as a 5 $\log_{10}$ reduction dose. Sommers and Thayer (*17*) found that D_{10} values for a mixture of four *L. monocytogenes* strains surface inoculated onto commercially available frankfurters ranged from 0.48 kGy to 0.71 kGy (Table III). Radiation doses of 2.45 to 3.55 kGy are therefore needed to eliminate 5 $\log_{10}$ of the pathogen from hot dogs. Niemira et al. (*18*) found D_{10} values ranging from 0.62 kGy to 0.77 kGy when *L. monocytogenes* strain H7762 was surface-inoculated onto beef frankfurters or soy-based imitation meat products (Table III). Foong et al. (*19*) found that doses ranging from 2.5 kGy to 3.0 kGy were required to eliminate 5 $\log_{10}$ of the microorganism from four different types of RTE meat products. Thayer et al. (*20*) found a D_{10} of 0.69 to 0.70 kGy for *L. monocytogenes* inoculated into cooked ground turkey meat or cooked turkey nuggets.

The role of specific additives on the radiation resistance of *L. monocytogenes* inoculated into RTE meats was investigated. Sommers et al. (*21*) found that soy protein concentrate, an extender that contains phytates and isoflavones with antioxidant activity, increased the radiation dose required to eliminate 5 $\log_{10}$ of the pathogen from 3.1 to 3.75 kGy. In other work, application of citric acid, a pH reductant and antimicrobial, to frankfurter

Table III. D_{10} Values of *L. monocytogenes* Inoculated onto RTE Meats

Product (Study)	*D_{10} (kGy)*	*Reference*
Beef Frankfurter #1	0.52	17
Beef Frankfurter #2	0.52	17
Mixed Meat Frankfurter #1	0.71	17
Mixed Meat Frankfurter #2	0.71	17
Poultry Frankfurter #1	0.49	17
Poultry Frankfurter #2	0.70	17
Poultry Frankfurter #3	0.64	17
Beef Bologna #1	0.60 – 0.62	21
Beef Bologna #2	0.66 – 0.71	22
Turkey Bologna	0.58	This Study
Cooked Turkey	0.68 – 0.70	20
Soy Hot Dog	0.77	18

surfaces increased the radiation sensitivity of *L. monocytogenes* (*8*). The inclusion of sodium diacetate, or sodium diacetate and potassium lactate mixtures, in the formulation increases the radiation sensitivity, and prevents post-irradiation growth, of *L. monocytogenes* inoculated onto cooked beef bologna (*9, 10*). The phenomenon of variability in the radiation resistance of *L. monocytogenes* on RTE meats could be reproduced using commercially used additives.

Mechanism of Lethality. Ionizing radiation induces DNA strand-breaks, transition mutations, transversion mutations, frameshift mutations, and deletions in bacterial cells (*22 - 25*). Mudgett et al. (*26*) observed that gamma radiation induced mutagenesis in *Escherichia coli* began when post-irradiation survival reached 1.5%, or a dose of 0.6 kGy. Wijker et al. (*27*) recommended 0.25 kGy, a gamma radiation dose that decreased survival to 2%, for proper selection and characterization of mutants in *E. coli* strain EC919. In *L. monocytogenes,* a radiation dose of 2.0 to 2.5 kGy is required for a 1 to 2% single gene inactivation rate, as determined by mutation of the microorganism's *hlyA* (hemolysin) gene (Figure 1). Ionizing radiation also disrupts cell membrane associated with DNA complexes that are required for plasmid partitioning and active sites for the DNA repair process (*28-31*), and also induces loss of plasmids that carry genes required for food-borne pathogen virulence (*32*). Disruption of the *L. monocytogenes* cell membrane by ionizing radiation can also lead to increased sensitivity to organic acids and antimicrobial compounds such as diacetate (*9*).

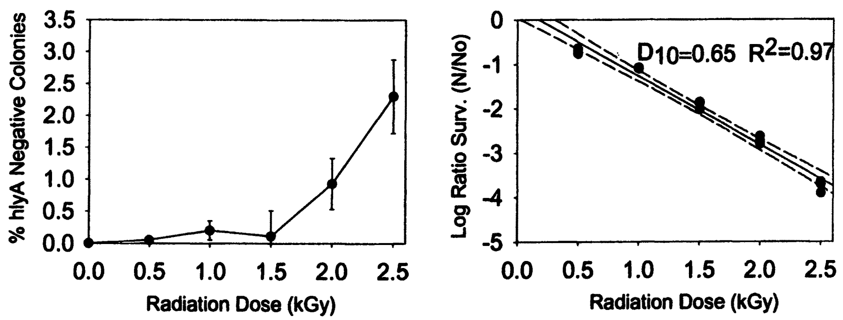


Figure 1. Radiation resistance and mutagensis of L. monocytogenes H7762 that was surface-inoculated onto beef frankfurters. Each experiment was conducted independently three times. Mutants were selected by plating on blood agar plates. Individual log$_{10}$ reduction points and 95% confidence limits are shown for the D$_{10}$ value while error bars are shown for hlyA mutation rate at each radiation dose (This Study).

Product Chemistry and Quality

Lipid Oxidation. Oxidation of lipids in meat products, an autocatalytic and temperature dependent process, is enhanced by the presence of oxygen, and can be induced by free radical generators including ultraviolet light and ionizing radiation (*33*). Ionizing radiation induced lipid oxidation also results in the formation of aldehydes, ketones and diacyglycerols (*33-36*). A number of studies have examined lipid oxidation in RTE meats. Radiation doses of 3 to 4 kGy result in a statistically significant doubling of lipid oxidation in beef bologna (15% fat) (*8-10, 21, 37*). Nam et al. (*38*) found increased lipid oxidation in precooked turkey, pork, and beef patties irradiated under aerobic conditions, which was ameliorated by vacuum packaging. Lipid oxidation in RTE meats can be influenced by product formulation. Du et al. (*39*) found that inclusion of antioxidants into the emulsion of sausages made with turkey leg meat lessened radiation induced lipid oxidation while Sommers and Fan (*37*) found that inclusion of excess glucose (>4%) increased the likelihood of lipid oxidation in beef bologna.

Volatile Sulfur Compounds (VSC's). Previous studies of irradiated raw meats have indicated that off-odors can be generated as a result of the irradiation process (*34, 35, 40, 41*). The off-odor has been called 'irradiation' odor, and has been characterized as 'wet dog', 'sulfide', 'metallic', 'wet grain', 'goaty' or 'burnt' (*41*). The changes in off-odors are primarily due to formation of volatile compounds including hydrocarbons, alcohols, aldehydes and ketones, which are generated from lipids (*38-45*). Several VSC's, derived from radiolysis of sulfur containing amino acids, include methyl sulfide, hydrogen sulfide, sulfur dioxide, dimethyl disulfide, methanethiol, (methylthio) acetic acid and carbon disulfide, are produced in irradiated raw and RTE turkey meat (*39,42,43*) (Figure 2). Turkey muscle is one of the meats most sensitive to irradiation in terms of off-flavor development (*46*). While these compounds can be produced in all meats, off odors have not been noted to be problematic following irradiation of beef and mixed meat fine emulsion sausages (*8-10, 21, 37*).

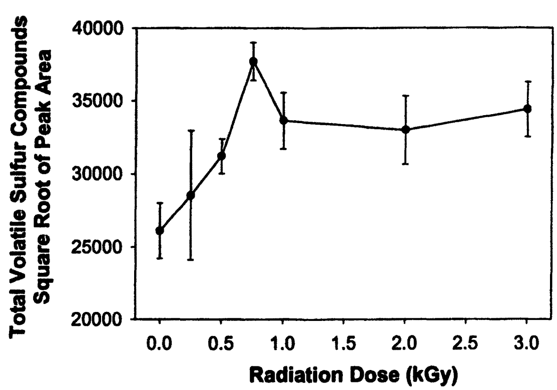


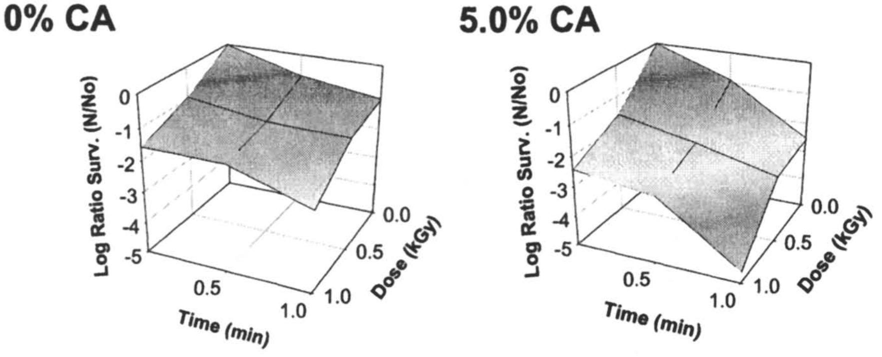
Figure 2. Generation of volatile sulfur compounds in vacuum-packaged RTE turkey meat by irradiation. RTE turkey meat as determined by pulsed flame photometric detection (PFPD) (43). Results are the average of nine (n=9) samples and represent the sum of hydrogen sulfide, sulfur dioxide, methanethiol, and dimethyl disulfide peak area square roots. Standard error bars are shown at each radiation dose (This Study).

Color. Ionizing radiation can induce color changes in RTE meat products (*47*). RTE poultry meat has been problematic for ionizing radiation induced color changes. In poultry, especially turkey, this manifests itself as a radiation induced increase in redness (a-value). Nam et al. (*38, 45*) suggested that carbon monoxide heme pigments could be responsible for the increased redness. As with VSC's, and other volatile compounds, the use of antioxidants and vacuum packaging were capable of reducing the induced color change (*39, 42*). Ionizing radiation has also been shown to induce loss of redness in beef bologna and frankfurters (*8-10, 21, 37*). As with lipid oxidation and VSC generation color change in irradiated meats can be influenced by product formulation, with loss of redness being enhanced by excess carbohydrate and lessened by inclusion of commonly used coloring agents such as paprika oleoresin (*8, 37*).

Elimination of *L. monocytogenes* versus VSC Generation. A number of approaches can be used to ameliorate the problem of VSC generation in RTE turkey meat. One such approach could include the use of antioxidants in the RTE meat formulation prior to cooking and irradiation (*39*). Another approach, as mentioned earlier, would be to include antioxidants in the diets of animals prior to slaughter (*42*). Still another approach is to lower the ionizing radiation dose required to eliminate *L. monocytogenes,* so as to not affect product quality.

Thayer et al. (*48*) demonstrated that application of heat following irradiation increased the $\log_{10}$ reduction of *Salmonella* on chicken meat. Sommers et al. (*8*) demonstrated that reducing the pH by use of a citric acid dip could increase the ability of ionizing radiation to eliminate *L. monocytogenes* from vacuum-packed frankfurters. Juneja and Eblen (*49*) demonstrated increased sensitivity of *L. monocytogenes* to heat in the presence of acidulant.

In this study, the use of citric acid applied to the turkey deli meat surfaces, ionizing radiation, and heat (75°C) was evaluated for the ability to eliminate *L. monocytogenes* H7762 and reduce the generation of VSC's. The ability of the combination treatments to eliminate *L. monoctyogenes* from the surface of RTE turkey is shown in Figure 3.



0% CA

5.0% CA

Figure 3. Elimination of L. monocytogenes H7762 from the surface of vacuum-packed RTE turkey meat by a combination of citric acid (CA) applied to the product surface, irradiation (to 1.0 kGy) and thermal treatment (75 °C). Each experiment was conducted independently three times (This Study).

The order of treatments was application of sterile deionized water or 5% citric acid to product surfaces (10 g pieces), vacuum packaging, irradiation to a dose of 1.0 kGy, and submersion in a water bath (75°C) for a period of 1 minute. The *L. monocytogenes* (10^8 CFU) was applied to the product surface following application of acidulant and prior to vacuum-packaging. Following treatments (acid, irradiation, heat, or combinations of the three) the samples were processed for microbiology (*8-10*). Each experiment was conducted independently three times.

Use of acidulant alone resulted in a 0.5 $\log_{10}$ reduction of *L. monocytogenes*. Use of irradiation in combination with heat, without acidulant, resulted in a 2 $\log_{10}$ reduction of *L. monocytogenes*. However, use of 5% citric acid (pH 4.5) in combination with irradiation and heat resulted in a 5 $\log_{10}$ reduction of the microorganism (Figure 3).

October 23, 2009 | http://pubs.acs.org
Publication Date: January 2, 2004 | doi: 10.1021/bk-2004-0875.ch005

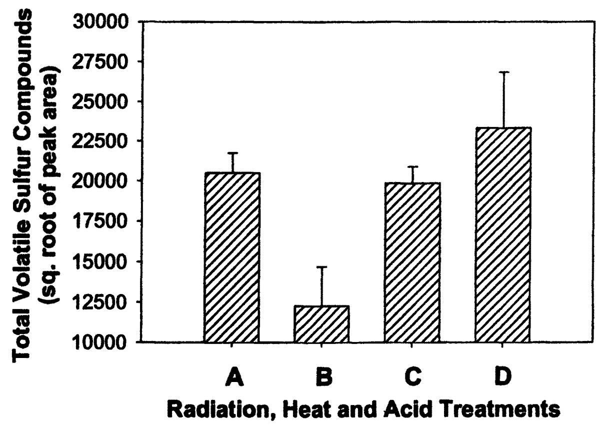


Figure 4. The generation of volatile sulfur compounds in irradiated (1.0 kGy), heated (75 °C for 1 min.), and acid treated (5% citric acid) ready-to-eat turkey meat. The treatments were (A) *Unirradiated, unheated and non-acid treated samples (B) Unirradiated, nonheated, acid treated samples (C) Irradiated, heated, and non acid treated samples (D) Irradiated, acid treated, and heated samples.* VSC's were *measured using pulsed flame photometric detection (PFPD) (15). Values represent the sum of hydrogen sulfide, sulfur dioxide, methanethiol, and dimethyl disulfide peak area square roots (n=9). Standard error bars are shown for each treatment (This Study).*

Following determination of the conditions required to eliminate 5 $\log_{10}$ of *L. monocytogenes* from the RTE turkey meat surfaces, the generation of VSC's was measured using uninoculated product. The treatment of 1 kGy, 0% acidulant, and 75°C for 1 minute reduced the VSC's to the 0 kGy level, possibly due to decreased thermal instability of the compounds. The reduction in VSC generation did not correlate with a significant reduction of *L. monocytogenes* (Figure 4). In contrast, the generation of VSC's was reduced significantly (ANOVA, n=9, α=0.05) from that obtained at the 1.0 to 3.0 kGy doses. No differences in product color or lipid oxidation were noted in the 5% citric acid, 1.0 kGy, and 75°C treated samples was noted.

Conclusions

What little data is available suggests that it should be possible to produce *L. monocytogenes* free, organoleptically acceptable, RTE meat products. Sensory studies of vacuum packaged frankfurters irradiated to doses of 8.0 and 30 kGy, at subfreezing temperatures, originally conducted for the purpose of providing rations for military personnel, have been conducted (*50-53*).

In those studies a radiation dose of 8.0 kGy produced undesirable sensory traits in 3 of 18 categories while frankfurters irradiated at a dose of 30 kGy were scored as being less palatable in 8 of 18 categories (*50-53*). Sensory studies conducted with vacuum packaged turkey frankfurters irradiated to doses of 5.0 and 10.0 kGy, at temperatures of 2°C and –30°C, indicated it was possible to obtain product which was not significantly different than non-irradiated frankfurters (*50-53*). While unknown at the time, the use of turkey frankfurters was important because of issues concerning volatile sulfur compounds generated by ionizing radiation. What then, is the threshold for generation of VSC's in actual products, as applies to consumer satisfaction, versus detection by analytical chemistry equipment by trained scientists? In more recent work Al-Bachir and Mehio (*54*) irradiated RTE beef luncheon meat to a dose of 4 kGy and found that the product was organoleptically acceptable, with the shelf-life being extended from 10 to 14 weeks.

The judge and jury for irradiated RTE meats will ultimately be the consumer. Unfortunately, despite a plethora of instrumental analysis of irradiated RTE meats, relatively little work has been published pertaining to consumer preferences for these products when irradiated to doses of <5.0 kGy needed for elimination of vegetative bacterial pathogens such as *L. monocytogenes* and *Salmonella* spp. Virtually no work has been done to correlate generation of specific volatile compounds, off-odor, off-flavors, etc. in RTE meats using modern formulations with consumer groups. Is there such a thing as irradiated (<5 kGy) consumer friendly turkey deli meat or hot dog? What is its formulation? What radiation dose will it tolerate? Will it be microbiologically safer? This promising area of research has a high potential for providing safer RTE meat products to consumers, yet many questions need to be addressed, especially the issue of RTE meat palatability versus formulation and radiation dose.

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Chapter 6

Improving Safety and Extending Shelf Life of Fresh-Cut Fruits and Vegetables Using Irradiation

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Irradiation can serve as a hurdle step in an overall safety plan that enhances safety while preserving quality of fresh-cut fruits and vegetables. Extension of shelf-life using irradiation is primarily due to the decrease in spoilage organisms, thus the effectiveness of irradiation depends on initial quality of the product. Irradiation at the levels optimal for shelf-life extension is also effective against pathogens found in fresh produce. Vegetative pathogens are destroyed while background flora is reduced but not eliminated. Combining irradiation with other technologies such as calcium treatment, warm water dips, and modified atmosphere packaging can further enhance shelf-life and mitigate adverse effects on quality.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Fresh-cut or minimally processed (MP) products are also referred to as "lightly processed", "partially processed", "fresh processed", and "pre-prepared." Fueled by demands for convenience and freshness, sales of ready-to-eat vegetables have grown rapidly in the last decade and are expected to reach $19 billion in 2003 (*1*).

Fresh-cut or minimally processed fruits and vegetables are defined as those trimmed, peeled or cut into 100% usable form and then packaged. The initial preparation and preservation treatments are often followed by some kind of modified/controlled atmosphere or vacuum packaging. Fresh-cut products are then subjected to low temperatures (above the freezing point) during storage, distribution, marketing, and just prior to preparation for consumption. Shredded lettuce, mixed salads, peeled carrots, cauliflower and broccoli florets, sliced mushrooms, sliced and diced tomatoes, cut bell peppers, and peeled garlic are examples of fresh-cut vegetables. Peeled and cored pineapple, peeled citrus fruits, sliced apples, cantaloupe chunks, and fruit salads are examples of fresh-cut fruits.

Shelf-life of Fresh-Cut Produce

Unlike most processing techniques that extend shelf-life, minimal processing increases perishability. Shelf-life of fresh-cut vegetables is generally 10-14 days and slightly less for fresh-cut fruit. The behavior of plant tissue that has been minimally processed is similar to that of tissue that has been wounded or stressed. This behavior includes increased respiration and ethylene production, and sometimes wound healing. Other consequences include oxidative browning reactions, lipid oxidation, and enhanced water loss. Several factors such as species and variety, stage of physiological maturity, extent of wounding, temperature, oxygen and carbon dioxide concentrations, water vapor pressure, and various inhibitors affect the intensity of the wound response. Microbial spoilage is also enhanced due to the presence of cut surfaces or damaged plant tissues that facilitate microbial colonization, active metabolism of the plant tissue, and methods used to extend shelf life that allow longer periods for microbial multiplication.

Microbiological Concerns with Fresh-Cut Produce

Fresh fruits and vegetables are often considered to be among the most healthful and safe foodstuffs available, yet, according to the Centers for Disease Control and Prevention, the number of produce-related outbreaks doubled between the period between 1973-1987 and 1988-1992 (*2*). Outbreaks with identified causes were primarily of bacterial origin with *Salmonella* topping the list (*2, 3*) although viruses (such as *Hepatitis A*) and parasites (such as *Giardia)* have also been implicated. Buck and others (*2*) note that enteric pathogens are among the greatest concern for fresh-cut fruits and vegetables because they have a low infectious dose and have the potential to grow on the product prior to consumption.

At any stage in the growth and production cycle of the product, the potential for contamination exists. The field or orchard, harvesting, sorting, washing, cutting, distributing and handling in the home or for food service all have the potential for introducing pathogenic organisms. Not surprisingly then, numerous isolations of a wide variety of pathogenic organisms from fresh fruits and vegetables have been documented (*2, 4, 5, 6, 7*). The increase in fresh produce consumption, the trend towards eating away from home, a proliferation of salad bars, centralized processing, and an increase in global trade have been suggested as possible reasons for the increase in the number of food borne outbreaks related to produce (*2, 8*). Increased scrutiny of outbreaks and improved detection methods may also be contributing factors.

Preservation of Fresh-Cut Fruits and Vegetables

There is much interest in the fresh-cut industry to find effective strategies to extend shelf-life and minimize contamination by pathogens. The most common methods used to preserve the quality of fresh-cut fruits and vegetables are temperature control, chemical sanitation, and modified atmosphere packaging. Maintaining low temperature is critical to reduce respiration rate, microbial growth, and other deteriorative reactions. However, psychrotrophic organisms such as *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Aeromonas hydrophila* are capable of growing at refrigeration temperatures used to store fresh produce.

Various sanitizers can be used to wash raw fruits and vegetables. Sodium hypochlorite, calcium hypochlorite, chlorine dioxide, chlorine gas, hydrogen peroxide, ozone, and organic acids such as lactic acid, acetic acid and peroxyacetic acid are approved by the US Food and Drug Administration (FDA) for food contact (*9*). Antimicrobial activity depends on the amount of sanitizing compound that comes in contact with microbial cells. The inaccessibility of sanitizers to microbial cells lodged in crevices and natural openings in the skin contributes to the lack of effectiveness of available sanitizers in killing pathogens.

Modified atmosphere packaging (MAP) is used widely for fresh-cut produce spurred in part by advances in packaging material. High CO_2 levels and low O_2 levels are highly effective in delaying spoilage but have minimal effect on some pathogens such as *Listeria monocytogenes, Yersinia enterocolitica*, or *Aeromonas hydrophila* (*10, 11, 12*). Furthermore, although the incidence or *Clostridium botulinum* spore presence is low (0.36% in one study of 1118 precut MAP samples (*13*)), the low oxygen conditions may allow toxin formation (*14,* *15*). However, toxin is usually found in samples considered spoiled but is infrequently detected in a sample that would be otherwise considered edible (*15*).

Irradiation can serve as a hurdle step in an overall safety plan that enhances safety while preserving quality of fresh-cut fruits and vegetables. This non-thermal treatment is highly effective against many pathogens found in fresh produce and offers the potential, in combination with other treatments, of improving shelf-life.

Effect of Irradiation on Fresh-Cut Fruits and Vegetables

Effect on Microorganisms

Irradiation like other physical processing can be applied in various ways, at various times, and at various intensities. The process and the product will determine the level of effectiveness of irradiation treatment. For example, a noted decrease in sensitivity to irradiation is shown at temperatures at or below freezing. Furthermore, the food matrix with variable water availability, differing ionic concentrations, oxygen concentrations, and other factors will affect the dose needed to achieve the desired sanitizing effect (*16, 17*).

Most food pathogens including *Campylobacter jejuni*, *A. hydrophila*, *Y. enterocolitica*, *L. monocytogenes*, and *E. coli* O157:H7, have a low tolerance for irradiation whereas the spores of pathogens such as *Clostridium botulinum*, *Clostridium perfringens*, and *Bacillus cereus* are more resistant. Few studies provide D-values (the dose needed to achieve a 90% reduction in counts) for various pathogens on fresh-cut produce, but the high moisture content would suggest lower D-values. For example, D-value of *L. monocytogenes* on endive is only 0.20 (*18*) and D-value for *E. coli* on various lettuce samples is less than 0.12 (*19*) but on alfalfa sprouts, the D-value for *Salmonella* and *E. coli* O157:H7 is higher, 0.5 kGy and 0.32 kGy, respectively (*20*). Since the level of contaminating pathogens in produce is usually low ($\leq 10^3$ CFU/g), low levels of irradiation (<1 kGy) can be expected to eliminate the threat under most conditions.

It is important that effective dose be considered on a product-to-product basis because significant differences are observed for the radiation sensitivity of bacteria inoculated on related but structurally distinct type of vegetables. Niemira and others (*19*) found that an outbreak strain of *E. coli* O157:H7 inoculated on the leaf surface of red leaf (D=0.119 ± 0.004) or green leaf lettuce (D = 0.123 ± 0.003) was significantly more sensitive to irradiation than the same strain inoculated on iceberg (D = 0.136 ± 0.004) or Boston lettuce (D = 0.140 ± 0.003). Further differences can be expected in matrices that vary in intrinsic and extrinsic characteristics. The combination of stressors can increase the effectiveness of the achieved reduction. For example, irradiation at 1 kGy dose reduced counts of *L. monocytogenes* inoculated onto sliced cabbage or sliced radish by 4-5 logs and the surviving *Listeria* cells were found to be more sensitive to low temperature storage than the non-irradiated cells (*21*).

Raw alfalfa sprouts infected with either *E. coli* O157:H7 or *Salmonella* spp. were responsible for twelve outbreaks between 1995 and 1999 resulting in an FDA recommendation to disinfect seeds with 20,000 ppm calcium hypochlorite. USDA researchers Rajkowski and Thayer (*20*) report that irradiation at 2 kGy dose decreased aerobic counts by 2-3 logs and coliform counts by 5 logs. While there was a dose-dependent effect on yield *(wt/wt)*, there was little effect on germination, structure of the sprouts was maintained, and shelf-life was extended by 10 days. In a different study, elimination of *L. monocytogenes* inoculated on alfalfa sprouts (6 logs CFU/g) required dose levels of 3.3 kGy or higher but there was no observed changes in appearance or odor (*22*).

Low temperature storage is necessary to ensure the safety of fresh-cut produce regardless of the preservation treatment used. *Shigella* (*23*), *Salmonella* (*23, 24*), and *E. coli* O157:H7 (*25*) have been shown to multiply easily in cut fruit products held at abuse temperatures (room temperature) conditions. This safety risk is underscored by a study conducted by Larson and Johnson (*26*) that demonstrated that melon cubes inoculated with *C. botulinum*, treated with ultraviolet radiation and stored in a passively modified atmosphere could support toxin formation when temperature was abused (27°C). The important point of this study was that inhibition of spoilage organisms with UV light might result in toxin formation in the absence of overt spoilage if the samples were temperature abused. Since spores are more resistant to chemical and physical treatments, it can be argued that any treatment that reduces the background flora is at risk for this general conclusion.

A small reduction and/or inhibition of germination might be expected for spores subjected to low dose irradiation. While studies on botulinum toxin production in fresh-cut fruits and vegetables irradiated at low dose levels are infrequent, valuable information can be obtained from studies assessing the combined effect of MAP and irradiation on meat based products. In a review of these works (*17*), Radomyski and others conclude that low dose irradiation does not increase the risk of sporogenous bacteria. The authors point out that, other processing technologies, notably thermal pasteurization, also fail to eliminate spores, and are accepted as effective tools for improving food safety.

Recovery of Organisms

Irradiation at low dose levels might not kill all cells but instead cause injury. Given appropriate conditions, these microorganisms can repair themselves and multiply. Lucht and others (*27*) found that incubating organisms at temperatures suboptimal for growth for a period of up to 18 h before transferring to growth temperatures could greatly enhance the recovery of radiation-injured organisms. This was especially true for *E. coli* and salmonellae but less important for *Listeria, Yersinia, Aeromonas,* and *Staphylococcus.* Similarly, organisms injured by heat, cold or acid could be recovered in greater numbers when they were allowed to incubate on non-selective media before being subjected to selective agents (*28, 29, 30*).

In an ongoing study where cilantro was contaminated with a cocktail of 6 strains of *E. coli* O157:H7 and then washed and irradiated at 0.5 kGy, we recovered a greater number of cells using a modified recovery protocol than when cells were plated directly to selective media (Figure 1). The modified recovery protocol employed both the Thin Agar Layer method (*28*) and a suboptimal growth temperature (*27*) of 18°C for18 h before being moved to 35°C.

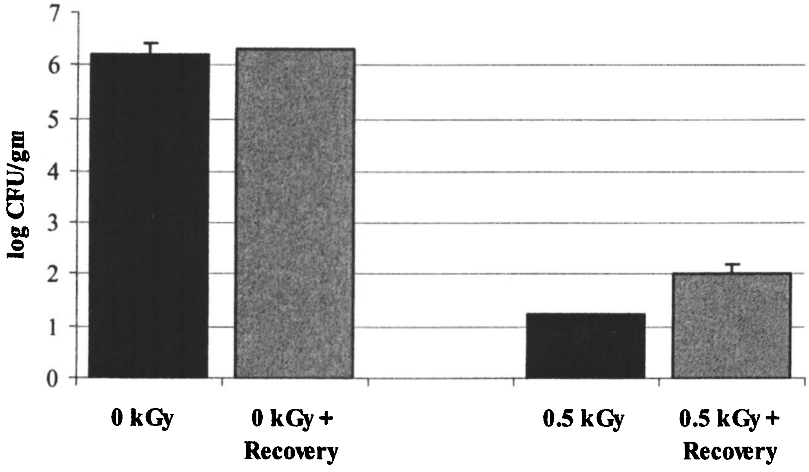

Figure 1. Increased recovery of E. coli O157:H7 using a Thin Agar Layer Method for contaminated cilantro after a chlorinated water rinse and 0.5 kGy gamma irradiation. Each point shows the mean and standard deviation of four replicates.

Shelf Life

The primary benefit of irradiation is in increasing the shelf-life of those products where shelf-life is limited by microbial action. Radiation at dose levels of less than 2 kGy can extend shelf-life by 2-12 days for several fresh-cut products. These include prepacked soup greens (*31*), fresh sweet corn (*32*), pico de gallo (*33*), shredded carrots (*34*), diced bell peppers and radish (*21*), celery (*35*), romaine lettuce (*36*), iceberg lettuce (*37, 38*), and diced tomatoes (*39*). Reduction by several logs were noted in aerobic plate counts, yeast and mold, lactic acid bacteria and Enterobacteriacae and in most cases sensory qualities such as color, flavor, texture, and odor were not affected.

An increase in shelf-life poses a question of safety should the product be contaminated. Pathogens generally do not affect sensory qualities and since the product continues to look, smell, and taste good, the increased shelf-life provides additional time for pathogens to multiply. However, irradiation at the levels optimal for shelf-life extension is also effective against vegetative pathogens found in fresh produce. Concerns about completely eliminating background microflora and thus providing an environment for outgrowth of pathogens have proven to be unfounded. Vegetative pathogens are destroyed at similar or greater levels compared to background flora and do not out-compete and outgrow these organisms. For example, our results for cilantro irradiated at 0.5 kGy showed that yeasts and mold were reduced by 3.7 logs and total aerobic counts by 3.3 logs but *E. coli* O157:H7, enumerated after a recovery protocol, was reduced by over 4 logs. Larger reductions for inoculated organisms of concern also occurred with both *E. coli* and *L. monocytogenes* on diced celery (*35*) and for *E. coli* O157:H7 on iceberg lettuce (*38*). In the presence of reduced competition, these pathogens do not grow at significantly greater rates compared to growth rates in non-irradiated samples. Furthermore, reliance on competitive microflora to ensure safety is not advisable. This is well illustrated by Barakat and Harris (*12*), who showed that the presence of background microflora had no effect on growth rate of *Listeria monocytogenes* and *Yersinia enterocolitica* in a modified atmosphere packaged poultry product.

It is also important to note that at low dose levels, growth of spoilage microorganisms is suppressed and delayed but spoilage occurs following a lag period (*21, 33, 34, 40*). Figure 2 shows the effect of 0.5 kGy irradiation on total plate counts in diced green onions. Irradiation initially decreased counts below the detection limit (50 cfu/g) but colonies were observed at day 4 and the counts increased steadily at a rate comparable to the non-irradiated control until the end of study on day 13. The risk of botulinum toxin production in temperature-abused samples has been discussed previously in this chapter. Provided treated product is held at appropriate temperatures, spoilage will occur before toxin formation commences (*17, 26*). Thus, it can be concluded that low dose irradiation does not increase the risk of vegetative or sporogenous pathogen outgrowth due to background flora reduction under proper storage conditions.

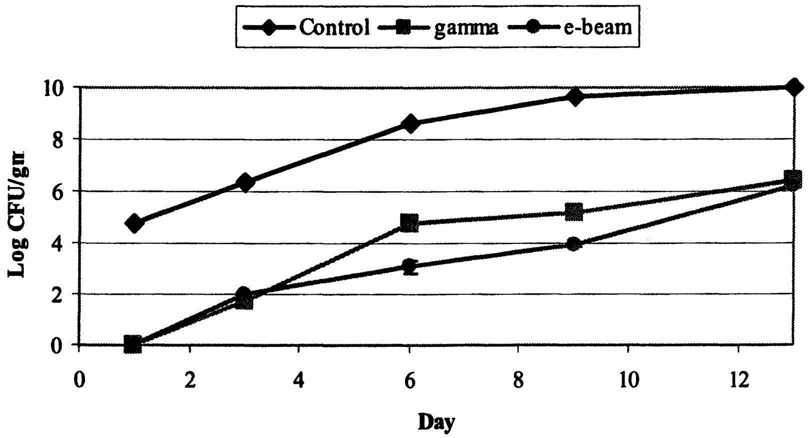

Figure 2. Total aerobic CFU/gm of chopped green onions subjected to gamma irradiation (1.1 kGy) or electron beam irradiation (1.0 kGy). Each point shows the mean and standard deviation of four replicates.

Wound Response

Irradiated fruits and vegetables can exhibit responses similar to those observed in wounded or stressed tissues. Depending on the dose level, responses may include increased respiration and ethylene production as well as physical and chemical reactions such as enzymatic browning, lipid oxidation, and loss of cellular integrity and enhanced moisture loss. Elevated ethylene levels stimulate respiration leading to increased oxygen uptake and release of carbon dioxide. These have direct effects by accelerating deterioration and senescence in vegetative tissues.

Couture and others (*41*) found that the rate of respiration increased linearly with dose (maximum of 4 kGy) but the effect was transient and the rate decreased back to pre-irradiation levels for the lowest dose, 0.3 kGy, within 24 hours. Ethylene production also increased reaching a maximum at 1 kGy. Gunes and others (*42*) found that irradiation of apple slices at dose levels of less than 1.2 kGy had no effect on rates of CO_2 production nor O_2 consumption, and irradiation at dose levels between 1.2 and 2.4 kGy had minimal effect. However, at higher dose levels (up to 11 kGy), the respiratory response increased with dose. They also observed that irradiation reduced ethylene production. Hagenmaier and Baker (*40*) observed that respiration rate of 0.19 kGy treated shredded iceberg lettuce increased by 33% as compared to the control one day after processing, was virtually the same 8 days following treatment, and was slightly lower after 13 days. At higher dose levels (0.5, 1, and 2 kGy), however, irradiation did cause a faster initial change in respiration rate of fresh-cut iceberg lettuce (*43*). In grated carrots, gamma irradiation at 2 kGy caused a 50% decrease in respiration and an 80% reduction in ethylene two days following treatment (*34*). In addition, irradiation was observed to delay senescence and reduce microbial spoilage.

These studies indicate that the change in respiration rate depends on the product and irradiation dose level, also that changes in respiration rate are usually transient lasting from an hour to several days. In some cases, ethylene levels decreased following irradiation and this could be a factor in extension of shelf-life observed for those products.

Effect on Enzymes

The direct effect of irradiation on enzymes is deamination and decarboxylation, thus affecting enzyme activity. Indirectly, irradiation can affect the integrity of cell membranes thus allowing substrates to come into contact with previously compartmentalized enzymes resulting in browning reactions and changes in texture.

Little information exists on the effects of irradiation on enzymatic activity specifically in fresh-cut products. In mushrooms, polyphenol oxidase activity was found to be reduced by irradiation at 0.5 and 1.0 kGy (*44, 45*). El Assi and others (*46*) found that whole tomatoes irradiated at 0.73 or 2.21 kGy doses had significant decreases in texture, attributed to depolymerization of pectin. They also determined that polygalactouronase (PG) activity was irreversibly suppressed but pectin methylesterase (PME) was significantly increased.

Another stress response to irradiation includes stimulation of phenol biosynthesis, particularly stimulation of phenylalanine-ammonia lyase (PAL), the first enzyme in the pathway for enzymatic browning. Hanotel and others (*47*) observed a 30% inhibition of polyphenoloxidase and peroxidase activities in cut witloof chicory following irradiation at 3 kGy. They attributed the increase in browning to an increase in phenolic metabolism, a reduction in antioxidant capacities, as well as to increased membrane permeability that would allow contact between the enzyme and substrate. Fan and others (*43*) found that irradiation at 1 and 2 kGy doses increased phenolic content of fresh-cut iceberg lettuce but browning was reduced which they attributed to the high levels of CO_2 in the package.

Effect on Quality Factors

The higher respiration rates of fresh-cut fruits and vegetables cause them to deteriorate faster compared to intact products, often with significant effects on quality factors such as texture, aroma and flavor. The effect of irradiation is varied; in some cases low doses can cause significant loss in firmness, in other fruits and vegetables no such loss is observed. Spoilage microorganisms also have negative effects leading to fermented odors, off-flavors, sliminess, and changes in appearance. Extension of shelf-life using irradiation is primarily due to the decrease in spoilage organisms thus the effectiveness of irradiation depends on the initial quality of the product. In a product with high counts of microorganisms prior to irradiation, extension of shelf-life will be less as compared to a product with low initial counts.

Texture

Texture is the quality factor most affected by irradiation. Most fruits are sensitive to irradiation even at low doses and the effect on vegetables is varied. Firmness of diced Roma tomatoes irradiated at 0.5 kGy decreased by 30% (*39*) and firmness of cut romaine lettuce irradiated at 0.35 kGy decreased by 10% (*36*). However, no change in firmness was observed in shredded carrots (*48*), fresh-cut iceberg lettuce following irradiation at 1 and 2 kGy (*43*), or in celery irradiated at 1 kGy (*35*). Textural softening attributed to irradiation has been linked to partial depolymerization of cell wall polysaccharides, cellulose and pectin with damage to cell membranes, as well as activation of pectinmethylesterase and inhibition of polygalacturonase, specific enzymes in the cell wall that act to solubilize pectic substances (*8*, *11*).

Flavor and Aroma

At dose levels used for fresh-cut fruits and vegetables, the effects of irradiation on flavor and aroma are minimal. An increase in sweetness in cherries (*49*) and a decrease in acidity of strawberries (*50*) have been reported. Fan and Sokorai (*51)* determined that irradiation up to 3 kGy had minimal effects on volatile compounds of cilantro and that loss of these compounds was more related to the 14 days storage. In diced bell peppers, however, irradiation at 3.7 kGy reduced bell peppers flavor and produced some off-flavors (described as cooked or stewed). This was in contrast to the control and the sample irradiated at 1.2 kGy, in which no effect on flavor or aroma was perceived (*52*). After storage for 9 days, off aroma was significantly higher in the control sample coinciding with a slimy appearance attributed to microbial spoilage. Thus, low dose irradiation has a beneficial effect on flavor and aroma by reducing the number of spoilage organisms that generate off-flavors and aromas.

Moisture Loss/Electrolyte Leakage

Radiation injury to cell membranes can result in the release of ions. This further results in the disruption of membrane functions such as permeability and activity of membrane-bound proteins, which causes electrolyte leakage and loss of moisture. For example, irradiation of cauliflower florets at 2 kGy damaged the microsomal membranes leading to extensive protein loss and an increase in electrolyte leakage (*53*). Similarly, a dose-dependent increase in electrolyte leakage was observed in fresh-cut iceberg lettuce irradiated up to 4 kGy (*37*). At dose levels above 2 kGy a soggy appearance was noted and correlated with sogginess and electrolyte leakage.

Color

Generally, there is little if any change in color following low dose irradiation of fresh cut fruits and vegetables. Alfalfa sprouts irradiated up to 2.57 kGy showed an increase in carotenoid content but no consistent effect on chlorophyll content or color (*54*). In cantaloupe chunks, loss of color has been observed *(55)*. This bleaching effect on the surface of cantaloupe chunks irradiated at 3 kGy was observed in packages with high O_2 levels, suggesting an oxidative bleaching of the carotenoid pigment. There was no change in color of diced bell peppers irradiated at 1.8 kGy, but at 3.7 kGy, an increase in brightness, decrease in greenness, and an increase in yellowness were observed *(52)*. No change in chroma or hue angle was observed in diced green onions irradiated at 1 kGy *(56)* or on diced tomatoes irradiated up to 3.7 kGy *(39)*.

Combination Processing

Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) can mitigate some of the deteriorative reactions such as increased ethylene production and respiration rates that may be accelerated by irradiation. Selection of appropriate packaging material and atmospheres specific to the fresh-cut product (as opposed to the intact product) is critical for MAP to work optimally. Irradiation in combination with atmosphere packaging has been found to be beneficial for several fruits and vegetables. Hagenmaier and Baker (*48*) evaluated the effect of irradiation (0.5 kGy) on the microbial counts of MA packaged and chlorinated shredded carrots. They found that irradiated and chlorine treated carrots had counts of 200 CFU/g after two days compared to 13,000 CFU/g for the nonirradiated control. The effect of irradiation (0.15 and 0.35 kGy) and MAP on the microbial load of cut romaine lettuce has been investigated in our laboratory (*36*). The combination of MAP and 0.35 kGy irradiation resulted in a 1.5-log reduction in total aerobic counts. Modified atmosphere packaging exerted a beneficial effect on the sensory qualities by preventing pinking, a quality defect caused by the action of polyphenol oxidase.

Concerns regarding botulinum toxin production in MAP products have been discussed above. Since botulinum spores can germinate when oxygen levels decrease to less than 1% (*57*), maintenance of minimum oxygen concentrations as well as strict temperature regulation are necessary to ensure the safety of MAP packaged products.

Warm Water Dips

Dipping whole fruits and vegetables in warm water is commonly used to control insect infestation and surface microorganisms, especially fungus. In fresh-cut fruits and vegetables, several studies have shown the benefits of reducing spoilage microflora, hence, increasing shelf-life. Inhibition of certain enzymes such as PAL and induction of heat shock proteins with resulting reduction in browning has also been observed. Fan and others (*51*) theorized that since irradiation is a stress, accumulation of phenolics and browning reactions could be enhanced. A warm water dip prior to irradiation would inactivate PAL and mitigate these effects. They found that a combination of warm water treatment (47°C) and irradiation at 0.5 or 1 kGy on fresh-cut iceberg lettuce resulted in minimal loss of texture, vitamin C, or total antioxidants but had better overall visual quality and less browning (Figure 3).

Calcification

Calcification of fresh fruits and vegetables is accomplished by dipping the fruit or vegetable into a calcium salt solution, such as calcium chloride, at an ambient or higher temperature for a predetermined time to increase firmness. Pectic substances with a low degree of methyl esterification bind easily with divalent calcium to form cross bridges. These calcium-pectin interactions help maintain the structural integrity and could reduce irradiation-induced softening.

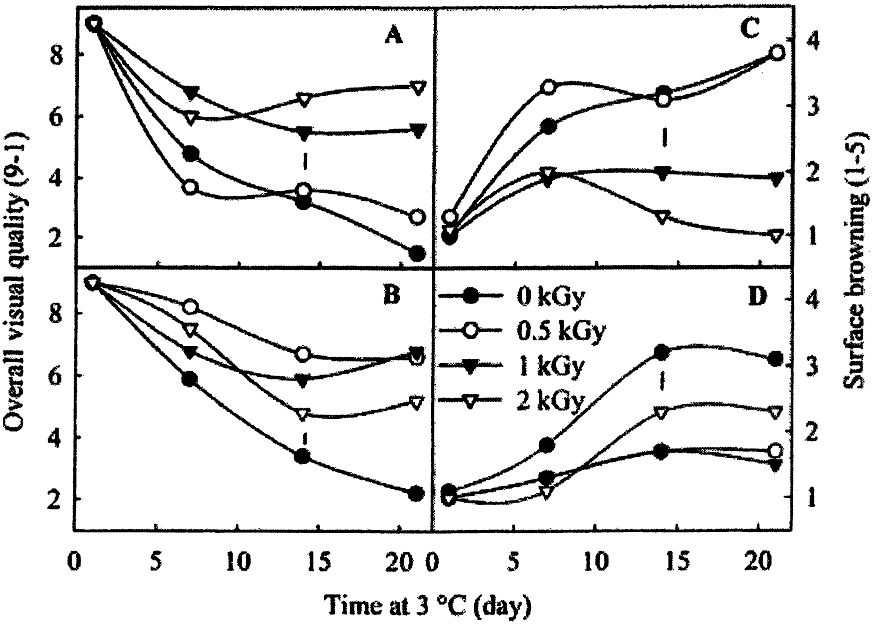

Figure 3: Changes in overall visual quality (A, B) and surface browning (C, D) of fresh-cut iceberg lettuce during storage at 3°C. Fresh-cut lettuce dipped in water of 5°C (A, C) or 47°C (B, D) was irradiated at a dose of 0, 0.5, 1, or 2 kGy. The samples were then stored at 3°C. Values are means of four replicates. Vertical bars represent the LSD (P<0.05) values. (Reproduced from reference 51. Copyright 2003 American Chemical Society)

For example, in apple slices, irradiation above a 0.34 kGy threshold decreased firmness, however, a calcium dip prior to irradiation prevented this irradiation-induced softening (*42*). Similarly, calcium concentration of diced Roma tomatoes was increased 3.2 fold using either a 1% calcium chloride or a 2% calcium lactate dip (*58*). Although irradiation at 1 kGy decreased firmness of the calcium treated samples, these irradiated samples were still significantly firmer than the not dipped but irradiated samples. After 8 days of storage at 4°C, diced tomatoes dipped in 1 or 2% calcium salts maintained firmness, while the control samples were considerably softer (Figure 4).

In addition to increasing firmness, calcium treatments have been shown to improve postharvest quality by decreasing ethylene production and reducing respiration with significant impact on shelf-life (*59*). These effects can be attributed to limiting the diffusion of substrate from the vacuole to the respiratory enzymes in the cytoplasm (*60*).

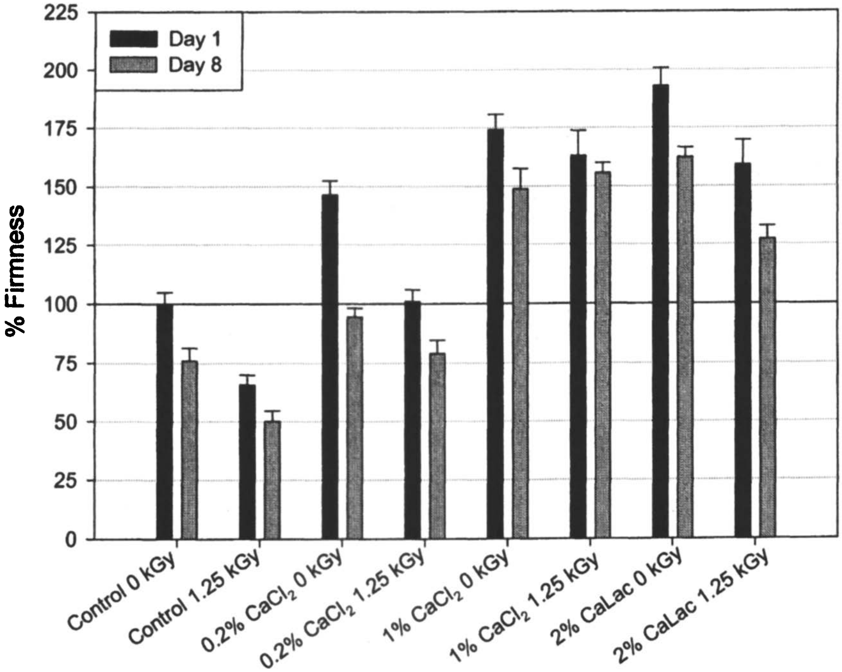

Figure 4: Effect of calcification and irradiation on the firmness of diced tomatoes. Fresh-cut diced tomatoes were immersed in either 1% calcium chloride or 2% calcium lactate and gamma irradiated at 1 kGy. Values represent the average six replicates. Error bars represent the standard error of the mean.

Conclusions

Certainly, some products are more suitable for irradiation than others, but the benefits of using irradiation for pathogen control and/or extension of shelf-life with minimal or no loss of quality are becoming increasingly clear. Combining irradiation with other technologies such as calcium treatment, warm water dips, and modified atmosphere packaging can further enhance shelf-life and mitigate adverse effects on quality. As with other products, adoption of this technology for fresh-cut fruits and vegetables will depend upon consumer acceptance and economic benefits. Other considerations include 1) evaluation of irradiation modalities such as x-ray and e-beam for fresh-cut produce in terms of penetration depth and dose uniformity, 2) regulatory approval of packaging material particularly suited for irradiated produce (including optimization of modified atmosphere packaging), 3) ensuring low temperatures during irradiation, and 4) legislative approval of irradiation for fresh-cut produce. While the advantages of irradiation for fresh-cut applications has been clearly demonstrated, commercial applications for produce remain to be exploited.

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Chapter 7

Ionizing Radiation of Seeds and Sprouts: A Review: Irradiated Seeds and Sprouts

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The sprout seeds used for human consumption are the suspect carrier of bacterial pathogens (*Salmonella* and *Escherichia coli* O157:H7), which can cause sprout related food borne illnesses. In 2000, irradiation to doses up to 8 kGy was approved for sprout seeds as a control. Literature exists on the effect of radiation to reduce pathogens on meat and poultry products; however, literature on pathogen reduction on seeds and sprouts by ionizing radiation is limited. Review of the research results on irradiated seeds indicates that each seed variety's germination and growth are affected differently by irradiation. Under the disinfection ruling, irradiation is approved up to 1 kGy for vegetable but not for pathogen reduction. There is a petition for ready-to-eat foods that would permit the use of irradiation to a maximum dose of 4.5 kGy on fresh sprouts. Research has shown that the irradiation process is a promising technology to increase shelf-life of fresh vegetables and sprouts and reduce bacterial pathogen contamination. Future areas of research are outlined for both raw sprout and sprout seeds to identify the approach of providing a safer product for the consumer.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Introduction

The production and consumption of sprouted seeds has increased worldwide due to the recognized enhanced nutritional values of sprouts over the seed (*1-3*). Seeds used for sprout production include: alfalfa, broccoli, radish, mung, soybean and some of the cereal grains (*4*). The seed sprouts can be eaten raw in salads, cooked in Oriental style meals, or squeezed to produce juice.

The food safety concern is that raw sprouts, when grown from contaminated seeds, can harbor human bacterial pathogens. Some human pathogens isolated from seeds or sprouts are: *Aeromonas* spp., *Bacillus cereus*, *Escherichia coli* O157:H7, *Klebsiella* spp., *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* (*5*). Production conditions for sprouts were reviewed by Price (*3*). The optimum conditions of moisture, temperature and relative humidity for seed germination and sprouting are the same conditions for microbial growth, including bacterial pathogens.

The ingestion of human pathogen contaminated sprouts can result in gastroenteritis or even death. A foodborne illness outbreak attributed to *Salmonella* occurred in Denmark, the United States and Canada when the same lot of contaminated alfalfa seeds was distributed internationally and then used to produce the sprouts (*6, 7*). In 1996-97, radish sprouts were the vehicle for a major *E. coli* O157:H7 outbreak in Japan, which resulted in over 10 deaths and 6000 illnesses (*8*). As a result of the increased sprout related outbreaks in the United States from 1996 to 1998, the Food and Drug Administration (*9*) issued consumer advisory warnings about the risk of eating raw spouts. The risks associated with the consumption of fresh sprouts were also outlined by Thompson and Powell (*10*). The FDA's warning states that children, the elderly and persons with weakened immune systems are considered at high risk and this population should not eat raw sprouts (*9*). When the warning was reissued, the news media in the United States did a feature on the risks of bacterial infection from eating raw sprouts (*11*). Updates of the status of any current reports of sprout related recalls or foodborne outbreaks can be obtained from the FDA's web site (http://www.cfsan.fda.gov) or FSNET (http://www.foodsafetynetwork.ca).

Microbiology

Seeds

The sprout seeds used for human consumption are now considered the main source of the pathogen contamination. These seeds can become contaminated in a variety of ways: 1) use of untreated manure and contaminated water source; 2) insect/bird/animal vectors; and, 3) unsanitary harvesting, processing, packaging, and retail conditions. The non-pathogenic aerobic microflora level on the seeds can range from log 2 to > log 7 CFU/g seed (*12, 13*). The human pathogen level on the seeds is low; however, these pathogens can survive for long periods (*14*). Under the conditions of sprouting (moisture and temperature), these pathogens can proliferate to levels that can cause foodborne illnesses if consumed (*10*).

Sprouts

The ecological factors influencing survival and growth of human pathogens on vegetables (sprouts) were reviewed by Beuchat (*15*). In addition to these factors, the fact that sprouts are grown hydroponically increases the risk of pathogen contamination. After 4 to 10 days growth in the warm, moist environment, the sprout can have an aerobic bacterial count ranging from log 3 to > log 9 CFU/g, depending on the geographic location of the facility and seasonal temperature (*16*). These high bacterial numbers represent harmless microflora, usually gram-negative rods identified mainly as *Pseudomonas* (*17*). Jaquette et al. (*14*) reported that under the sprouting conditions, if *Salmonella* is present, the pathogen can increase by 3 log CFU/g during a 24 h germination period. As a result of this increase of pathogen levels during the first 24 h of sprouting, the sprout water is now tested for pathogens according to the FDA's guidance to the sprout industry (*18*).

Interventions

Seeds

In order to reduce the risk of pathogen growth during sprouting, chemical and/or physical intervention techniques have been suggested to decontaminate the seeds. In order for the chemical treatment to be effective, the chemical disinfectant must: 1) reach and inactive the pathogen; 2) not adversely affect the seed viability; and 3) must be approved for food use. Treatments studied include disinfecting with such compounds as ethanol, hydrogen peroxide, ozonated water, calcium and sodium hypochlorite, and commercial products (*5*). Charkowski et al. (*19*) determined that wrinkled surfaces of alfalfa seeds contributed to increased aerobic counts after sanitizing the seeds prior to germination. They reported that wrinkled alfalfa seeds were more difficult to disinfect and recommended the removal of these wrinkled seeds as a means of lowering the risk of human pathogens in the seed lots. It is also suggested that the pathogen may be located within cracks or breaks in the seed coat. In their study using ozonated and chlorinated water, Rajkowski and Rice (*20*) reported that the hydrating alfalfa seeds released organic material into water, reacted with the disinfectants reducing levels, and increased the assimilable organic carbon levels in the sprout water. In order to reduce the risk of pathogens from the seeds in the sprouting process, it is recommended that seeds be treated with a 20,000 ppm calcium hypochlorite solution (*16*).

Irradiation up to doses of 8 kGy is approved for use on sprout seeds intended for human consumption (*21*). For this disinfection process to be effective the above three conditions must be met. With the irradiation process there is penetration of the energy particles. Any pathogen cells in cracks or under the seed coat can be killed by irradiation, provided that the dose level required to kill the pathogen does not affect the seed. Rajkowski and Thayer (*22*) and Rajkowski et al. (*23*) reported that a dose up to 2 kGy for irradiated alfalfa and broccoli seeds did not adversely affect the yield ratio and these seeds were acceptable for use by sprout growers. However, when Thayer et al. (*24*) and Rajkowski et al. (*23*) determined the D-radiation values, the radiation dose required to produce a 1-log reduction for *Salmonella* and *E. coli* O157:H7 on the dry seed they found that the pathogens were more resistant than previously reported on moist products. Using produce related outbreaks isolates, the D-radiation values for *Salmonella* on the dry alfalfa and broccoli seeds were 0.97 and 1.10 kGy, respectively, and for *E. coli* O157:H7 were 0.60 and 1.11 kGy, respectively (*24, 23*). The use of the irradiation process for pathogen reduction would only obtain about a 2-log reduction, which is similar to the chemical disinfection process (*5*). In order to obtain the 5-log reduction required (*16*), the dose needed would adversely affect both the germination and yield ratio for alfalfa and broccoli seeds.

Sprouts

Sprouts are fragile. Excessive manipulation reduces their keeping quality due to cellular leakage, which can support spoilage microorganisms, including pathogens. Chemical disinfection treatments of the alfalfa sprouts were reported using aqueous ozone, chlorine dioxide, hypochlorite commercial iodophor and Citricidal® (*25, 26*). None of these treatments were effective in eliminating the pathogens. Gagné et al. (*27*) reported that bacteria could reside in the xylem of alfalfa roots. When the bacteria pathogen is internalized, liquid disinfectant applied to the surface of the plant cannot reach them. In addition to the possibility of being internalized, the bacteria were shown to exist in biofilms on the sprout surfaces. Fett (*28*) suggested that the biofilm on sprouts may provide protection from any disinfection treatment.

Potential use of the irradiation process on fresh produce was reviewed by Thayer and Rajkowski (*29*). A maximum dose of 1 kGy can be used for disinfection, inhibition of sprouting (potatoes), and alteration of the maturation rate for greater shelf-life. The keeping quality of cut celery, bell peppers and carrots was increased after a 1-kGy treatment with gamma irradiation (*30, 31*).

In addition to increasing the shelf-life of the produce used in their studies, *L. monocytogenes* levels were reduced. Rajkowski and Thayer (*22*) reported a 2-log/g reduction in aerobic and total coliform counts after a 2-kGy treatment of alfalfa sprouts. As a result of this reduction the shelf-life of alfalfa sprout was increased by 10 days.

Current regulations do not permit the use of irradiation to control foodborne pathogens on sprouts. However, there are studies reporting the effectiveness of reducing the pathogens on sprouts using irradiation. Schoeller et al. (*32*) reported that a beta radiation dose of 3.3 or 5.3 kGy was effective in eliminating 6 logs of *L. monocytogenes* on alfalfa sprouts. Using gamma radiation, the D-values for *Salmonella* and *E. coli* O157:H7 inoculated on radish sprouts were 0.46 and 0.30 kGy, respectively (*33*). Elimination of the three pathogens at a 6-log/g level would be possible at the 3.3 kGy dose level. The ionizing irradiation process is a promising technology to improve the safety of food sprouts.

Germination and Yield of Irradiated Seeds

In order for the irradiation process to be used on seeds there can be no adverse effect on the germination, yield (growth), or nutrient content of the seed/sprout. Seed germination is the emergence and development from the embryo of the root, stem and primary leaf structures needed to produce the plant, and is measured in percent (number of germinated seeds/number of seeds. Yield (ratio) for the seed is the weight of the resulting sprout after a given growth time divided by the weight of seeds used (weight of wet sprout/weight of seed) and is used by the sprouting industry to determine the economic value of the seed.

For some seeds used for sprouting, the irradiation process reduces the percent germination and is dose related. Each seed variety responds differently. A reduction in percent germination of canary grass seeds by X-ray radiation was observed at doses of 0.2 and 0.3 kGy, but not at 0.1 kGy (*34*). When lentil seeds are gamma irradiated to 0.1 kGy, Chaudhuri (*35*) found no significantly different from the control. They reported that at 1.0 kGy, lentil seeds did not germinate. Soybean seeds are even more sensitive to radiation, 0.01 kGy gamma rays can reduce germination rate by 21% (*36*). Rice seeds are more resistant to gamma radiation and at 5 kGy, all rice seeds with intact hull had germination rates of 71-86% (*37*). Rajkowski and Thayer (*23*) found gamma irradiation of alfalfa seeds had little effect on germination at doses up to 2 kGy. In other instances germination was stimulated by the irradiation process. Germination of barley and corn was improved slightly by doses up to 8 kGy gamma radiation (*38, 39*).

The yield ratio or growth rate of the sprout is affected by the irradiation process. Shamsi et al. (*40*) found that very low doses (0.01- 0.03 kGy) gamma radiation of sunflower seeds stimulated vegetative growth. However, gamma irradiation (0.75 kGy) of mung bean seeds reduced the growth (length) of sprouts (*41*). In a comparison study between gamma and soft electron beam source, Kikuchi (*42*) reported that germination of soybeans was reduced at 5 kGy (gamma), whereas, the soft electron beam (60 keV, 26 kGy) has no effect. Kikuchi (*42*) found that there was a significant difference in the root length of the soybeans between the two irradiation processes. Fan et al. (*43*) and Rajkowski and Thayer (*23*) also reported shorter roots for alfalfa sprouts grown from gamma irradiated seeds at a dose > 2 kGy compared to those from non-irradiated seeds. The yield ratio was still acceptable for the alfalfa seed irradiated at 1 and 2 kGy (*23*). The results suggest that the growth rate of sprouts from irradiated seeds was slower, but lengths (yield ratio) of sprouts similar to those from non-irradiated seeds can be achieved if the sprouts were allowed to grow an extra 1-2 days, or if radiation doses are 2 kGy or lower (*23*). Chaudhuri (*35*) found that the yield of lentil seeds was markedly reduced and considered any dose >0.1 kGy as the critical dose that significantly inhibited the yield after 5 days growth.

It appears that very low-dose radiation stimulates the growth of some seeds. But high-dose radiation reduces germination rates, growth and yield. Many factors may affect responses of seeds to irradiation including type of seeds, varieties, seed coat composition, water content of seeds, and maturity of seeds. Further research is needed to determine the irradiation dose for each seed variety that affects both germination and yield ratio.

Nutrition Quality

Consumption of fresh vegetables, including sprouts, is associated with lower rates of cancers, and cardio- and cerebro-vascular diseases. Their consumption is also associated with lower mortality rates. The protection that vegetables provide against these diseases has been attributed to endogenous antioxidants. Antioxidant capacity of sprouts is among the highest vegetables that have been tested (*44*). Ascorbic acid (AA) is one of the antioxidants commonly found in fresh vegetables. Some of the sprouts have AA several times higher than lettuce (*45, 1*). Fan et al. (*43*) raised sprouts from non-irradiated and gamma irradiated (up to 3 kGy) alfalfa seeds and stored the sprouts at 7 °C for 21 days. They found sprouts grown from irradiated seeds had increased vitamin C (ascorbic acid plus dehydroascorbic acid) content and antioxidant capacity as measured by the ferric reducing antioxidant power assay, both initially and after 21 days. Fan and Thayer (*46*) gamma irradiated alfalfa sprouts and stored the samples at 5 °C for up to 14 days. They found antioxidant capacity of sprouts increased linearly with radiation dose at both 1 and 7 days of storage. Irradiation had minimal effect on vitamin C content when compared with the decrease in vitamin C content during storage. Carotenoid content of sprouts irradiated at 1.7 and 2.6 kGy was higher than that of control at 7 days of storage (*46*). Machaiah et al. (*41*) found low dose gamma radiation (0.25-0.75 kGy) of seeds improved the digestibility and nutritional quality of mung beans by reducing the content of oligosaccharides responsible for intestinal gas production. Ionizing radiation also reduces other anti-nutritional factors, such as protease inhibitors, in a number of seeds (*47*). The reducing sugars, mainly glucose, fructose and galactose, of sprouts, were enhanced by irradiation of seeds (*41*). Fan et al. (*43*) also noticed that irradiation of alfalfa seeds increased soluble solids content of the sprouts. Therefore, irradiation of seeds and sprouts at low doses did not negatively affect the nutritional quality of sprouts. For many seeds and sprouts, nutritional quality is significantly improved.

Sensory Quality

Gamma irradiated (5.0 kGy) and non-irradiated mung beans had no significant difference in their sensory acceptability (*48*). Levels of chlorophyll and carotenoid were similar in sprouts grown from irradiated and non-irradiated seeds, both initially and after 21 days storage at 7 °C (*43*). Chlorophyll and instrumental color parameters of alfalfa sprouts treated at doses up to 2.6 kGy gamma rays were not significantly different from non-irradiated sprouts (*46*). Sensory attributes of fresh and cooked mung bean sprouts were acceptable after irradiation of seeds to 0.75 kGy (*41*). Irradiation of alfalfa sprouts with beta radiation at 3.3 or 5.3 kGy caused no noticeable changes in appearance and odor while eliminating *Listeria monocytogenes* from inoculated sprouts (*32*). Generally speaking, fresh vegetables and sprouts can tolerate up to 1 kGy gamma radiation without significant changes in sensorial quality (*49, 50, 30*).

Conclusions

The use of the irradiation process (gamma and beta) affects each seed variety's germination and yield differently. The major concern with using the irradiation process is the inhibition of seed germination. Further studies using low-energy electrons are needed for each variety to determine the effect on germination and yield. In addition, the use of irradiation at low level in combination with chemical disinfection is another area of research that requires investigation.

The use of irradiation to increase the keeping quality of cut vegetables and sprouts while eliminating microflora is promising. There is a petition before the FDA (*51*) for approval to use a maximum irradiation dose of 4.5 kGy on ready-to-eat foods, which would include sprouts. With approval of this petition for use as a means of pathogen control, the irradiation process can be applied to provide a safer supply of fresh produce.

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Chapter 8

Irradiation Applications to Improve Functional Components of Fruits and Vegetables

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Fruits and vegetable are part of our daily diet and it is important to understand the role of postharvest treatment effects on functional components including organoleptic characteristics. Irradiation has multiple benefits in food preservation through several processes such as disinfestations, delaying maturation, sprout inhibition, decontamination, and sterilization. Sensory evaluation studies in different commodities indicate that irradiation treatment does not affect quality and flavor. Quality retention, along with efficacy and efficiency, is critical for many postharvest treatments. Although irradiated fruit retained quality compared to control, very little information is available on the effects of irradiation on functional components. Accumulative evidences from epidemiological, case and cohort studies have shown that functional components such as flavonones, flavonols may prevent chronic diseases such as cancer and cardiovascular diseases. In this chapter, emphasis is given to onion and grapefruit to illustrate the effects of irradiation on functional components. In onion, our results have demonstrated that irradiation at 0.8 and 1.2 kGy doses significantly increased both free and total quercetin concentrations. During the last four decades, ionizing radiation has been used as a quarantine treatment for eight fruit hosts that are shipped from Hawaii to the USA mainland. Flexibility and effectiveness of irradiation as a quarantine treatment have been demonstrated and proven to be appropriate for tropical fruits crops. However, several recent studies indicated the need of irradiation as an alternative for quarantine treatment in citrus to prevent infestation of Mediterranean (*Ceratitis capitata* (Weid), Mexican (*Anastrepha ludens* (Loew), and Carribbean (*A. suspensa* (Loew)) fruit flies. Our studies, in citrus, showed that low doses of ionizing radiation significantly increased flavonone concentrations. Potential use of irradiation to enhance the levels of functional components is discussed. To remain competitive in international and national markets, optimization of these components may be important for the processing industry.

Introduction

Irradiation has been used in food industry for several purposes. Approximately, 40 different irradiated food products have been cleared by 28 countries; some countries such as Netherlands are approving 20 different foods. Irradiation sources are mainly from gamma rays (cobalt-60 and cesium-137), machine generated electron beams (β particles), and X-rays. While X-ray radiation is concentrated in the same direction as electron beams, γ–rays from isotopes are emitted in all the directions uniformly (*1*). Traditionally, irradiation dose was measured in 'rad' (1 rad= 0.01Gy) but it has been discontinued, and the current unit of measurement is gray (Gy) and it is equal to the absorption of 1 J/kg. All the irradiation sources including Cobalt-60's gamma energy can penetrate food, causing small harmless molecular changes to the food.

The primary effects of ionizing radiation are ionization, dissociation, and excitation. When ionizing radiation passes through food, it loses energy (energy is absorbed). This absorbed energy or absorbed dose leads to ionization or excitation of atoms and molecules of the matter. Further it leads to chemical changes known to occur when food is irradiated. Free radicals produced as a result of these primary effects of ionization will lead to secondary effects that may interact with water to produce free radicals, which can diffuse far enough to reach and damage different important compounds of plant cell (*2*). Furthermore, radiolysis of water is therefore important in plants with higher water content because of its influence on temperature, pH, and dilution of solution by the presence or the absence of oxygen (*3*). However, the rise in temperature associated with radiation is minimal, and adverse changes in the food such as altered flavor, odor, color, texture and loss of nutritional quality are minimized (*4, 5*). Since there is no significant rise in temperature during irradiation, it is called as 'cold process.'

Consumer acceptance of irradiated food is a major issue for the food industry. While there are claims that gamma rays would change the chemical structure of food and produce unique radiolytic products (chemicals) that might prove harmful are still in consumers mind, accumulative studies have demonstrated to the US Food and Drug Administration (FDA) that no significant difference exists between irradiated and nonirradiated foods as far as safety is concerned (*6*). Initially, many investigations have been undertaken to study the potential use of ionizing radiation for several benefits like inhibition of sprouting (*7, 8, 9, 10*), disinfecting onions (*7*), and improving the storage quality (*10, 11*).

Under the permitted irradiation doses, fresh onions did not show any significant changes in the aroma constituents, which are the most unstable flavor components in onion (*12*). Sensory quality of irradiated bulbs was observed to be better than unirradiated ones (*13*). Although there were doubts about the prospect for using gamma irradiation as a postharvest treatment for fresh produce (*14*), the FDA has approved the treatment of fruits and vegetables with gamma irradiation up to 1 kGy dose (*15*).

Although irradiation was proposed as a treatment for fruit susceptible to fruit fly infestation in 1956 (*16*), only in the last four decades it has been used as a quarantine treatment for several fruits such as citrus (*17, 18*), mango (*19, 20, 21, 22*), and papaya (*23*). Gamma irradiation is used for quarantine treatment to control several fruit flies such as Oriental fruit fly (*24*) and Mediterranean fruit fly (*25, 26, 27*). Minimum absorbed doses suggested by USDA-APHIS for quarantine security of different fruits flies vary from 150 Gy for Mexican fruit fly (*Anastrepha ludens* (Loew)) to 250 Gy for Oriental fruit fly (*Bactrocera dorsalis* (Hendel)) with different geographical distribution (*28*). These irradiation doses for the same insect vary for each host crop. For example, a speculative dose for achieving quarantine security of Mediterranean fruit fly third instars varies from 70 Gy for grapefruit in Brazil to 215 Gy for papaya in the USA (*1*). Although several species of *Anastrepha* and *Bactrocera* probit 9 efficacy can be achieved with 60-100Gy, additional research has been suggested to determine whether the Mediterranean fruit fly and Oriental fruit fly can be controlled with <250 Gy (*1*).

Irradiation Effects on Functional Components

Recent cell culture and animal studies provide strong evidence that functional compounds derived from fruits and vegetables may play a chemopreventative role against cancer (*29*). Epidemiological and clinical studies are providing strong evidence that these functional components are responsible for a chemopreventive and therapeutic role in human health as demonstrated in large cohort and case-control studies in cancer and related diseases (*30-51*). The National Cancer Institute (NCI) estimates that one of three cancer deaths are diet related and that eight of ten cancer cases have a nutrition/diet component. These studies have prompted the NCI to recommend that people increase their dietary intake of certain foods, with citrus fruits being one of the food classes targeted by the NCI. Flavonoid-containing foods may prevent both heart disease (*45*) and cancer (*41, 42, 44, 48-50*), which are the two leading causes of morbidity and mortality in the United States. In this chapter, major emphasis is given on the influence of irradiation on flavonoids in citrus and onion and their biological activities. Furthermore, irradiation effects on other compounds such as carotenoids, pectin, vitamin C, quality, and sensory evaluation are also discussed.

Flavonoids

Flavonoids are widely distributed in the plant kingdom and are known to have strong antioxidant activity (*52, 53*).. Approximately, 8000 flavonoid structures have been identified (*54, 55*). The six major classes of flavonoids are flavones, flavonols, flavonones, catechins or flavan-3-ols, and isoflavones (*56*). Flavonoids, as a secondary metabolite, contribute to the plant's growth, reproduction and the morphology via pigmentation, as well as its resistance against pathogens and predators (*54, 57*).

In the USA, people consume about 1 g of flavonoid daily, while in some cultures this may be as high as 2-3 g per day in the diet (*58*). For a flavonol called quercetin, intake in Netherlands was estimated to be approximately 16 mg kg^{-1} (*59*); however, the total of flavonol and flavone is approximately five-fold lower than the previous findings of Kuhnau (*60*).

The basic structure of flavonoids is a C6-C3-C6 carbon skeleton. Each C6 represents an aromatic ring that commonly carries various substituents, like hydroxyl groups. The C3 carbon chain may have different oxidation levels and other structural features, which give rise to the differentiation among flavonoid classes (*61*). Flavonoids most commonly occur as glycosides, in which one or two sugar molecules are attached to the hydroxyl group (*54, 55*). In plants, flavonoids are produced via the shikimate pathway. The two C6 phenyl rings are commonly named A and B. The C3 chain usually produces an oxygenated cyclic structure and it is named C (*62*). Phenylalanine is a precursor of the B and C rings. Phenylalanine in plants is produced from the condensation of erythrose (derived from the pentose phosphate pathway) and phosphoenolpyruvate (derived from glycolysis). Phenylalanine is converted to cinnamic acid, later to coumaric acid (commonly 4-coumaroyl-CoA), which then is used for formation of the B and C rings (*62, 63*). The enzymes responsible for the conversion of phenylalanine into the B ring precursors are phenylalanine ammonia-lyase (PAL), cinnamate hydroxylase, and p-coumarate:CoA ligase. These three enzymes activities are increased by light treatments, especially that of PAL (*63*). The formation of flavonoid glycosides is positively affected by light (*64*). In parsley, light exposure triggered expression of flavonoid biosynthesis (*65*). Lees and Francis indicated that gamma irradiation exerts its positive effect on the flavonoid biosynthesis at an earlier metabolic point rather than the formation of these flavonol compounds (*66*).

Flavonols in Onion

Onion consumption has increased due, in part, to the presence of pharmaceutically important compounds such as quercetin and organosulphur compounds (*67*). There is a considerable geographical and cultural variation of dietary source of quercetin. For example, in Italy the main source is red wine, in China the main source is tea, and in the US and Northern Europe the main source is onion (*68*).

Flavonols are abundant in flower plants, leaves, seeds, and fruits, and usually give a pale yellow coloring. Another characteristic of this group of compounds is the double bond present in ring C (*60*). Major flavonols present in fruits and vegetables are quercetin, kaemferol, and myristin. Hertog and co-workers analyzed different fruits and vegetables for their quercetin content and an average amount reported was 15 mg/kg (*40*). In our study, red and yellow onion was shown to have higher levels of quercetin while white onions have negligible amounts of quercetin (*69*).

Different quercetin glycosides present in onions include quercetin 3,4' diglycoside, quercetin 7,4' diglycoside, and spiraeoside (quercetin 4' monoglycoside) (*70, 71, 72, 73*). It was previously believed that quercetin glycosides are not absorbed in the small intestines; only quercetin aglycone can be absorbed. Because most fruits and vegetables have higher levels of quercetin glucosides rather than its aglycone, researchers have attempted to enhance bioavailiblity by using enzymes (*72, 74*) and hydrochloric acid (*69*). Although the majority of quercetin is present in onions as pharmacologically less active glycosides, glycosidases produced by the human intestinal flora "fecalases" are capable of hydrolyzing a wide array of these quercetin glycosides to yield the aglycone quercetin (*75, 76, 77*). In view of the tumor inhibiting properties, partial or complete hydrolysis of glycosides can occur either by irradiation or various glucosidases present in the human gut, mouth, and feces (*72, 74*). Hydrolysis of quercetin glycosides using hydrochloric acid (HCL) provided 99% conversion of glycosides to aglycone (*69*). In order to convert different glucosides of quercetin, several postharvest treatments were theorized but a few treatments that expose the plant to stress were attempted. Treating grapefruits with irradiation was one of the treatments tested. Of eleven (different colored) cultivars of onions analyzed, aglycone content increased significantly (P=0.05) in diced onions treated with both 0.8 and 1.2 kGy doses in 'Cardinal', 'Dorado', and '20352-G' (Table I). The significant increase in aglycone content by irradiation could be due to partial hydrolysis and/or autolysis of quercetin diglycoside, yielding aglycone and/or stimulation of specific enzymes in the flavonoid biosynthesis pathway (*78*). Furthermore, irradiation treatment significantly (P=0.05) increased aglycone content in 'Cardinal' onion bulbs at 0.8 kGy compared to non-irradiated samples, and the aglycone content increased less at 1.2 kGy compared to 0.8 kGy (Figure 1).

Table I. Effect of Gamma Irradiation on the Aglycone and Total Quercetin Content in Diced Onions (mg/kg fresh wt)^{Z}

		Quercetin	
Cultivar	*Dose (kGy)*	*Free*	*Total (Free and glycosides)*
Red onions			
Cardinal	0.0	0.73^{b*} ± 0.57	164.76^{a} ± 26.97
	0.8	13.60^{a} ± 2.06	229.06^{a} ± 36.18
	1.2	12.77^{a} ± 2.15	179.09^{a} ± 31.92
Pink onions			
20352G	0.0	0.17^{a} ± 0.01	130.40^{a} ± 13.06
	0.8	15.15^{a} ± 5.60	167.89^{a} ± 18.83
	1.2	5.75^{a} ± 2.98	146.47^{a} ± 15.86
Yellow onions			
Dorado	0.0	0.15^{b} ± 0.01	132.86^{b} ± 10.4
	0.8	15.40^{a} ± 3.62	187.13^{a} ± 28.43
	1.2	12.28^{a} ± 3.35	160.04^{ab} ± 16.8

[*] Means in a column followed by the same letter (a, b) are not significantly different at P = 0.05. [Z] Results are given as means of 5 bulbs ± standard error (S.E.)

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Interestingly, many *in vitro* studies showed that quercetin glycosides are being absorbed in the small intestines. Furthermore, absorption of quercetin glycosides is relatively higher than aglycones'. Data have shown an association of a sodium-dependent glucose/galactose transporter called SGLT1. Gee et al. demonstrated that when rat jejunal segments were incubated with quercetin and quercetin glycosides, uptake of galactose and glucose was inhibited; the inhibition was greater with glycosides than with aglycones (*80*).

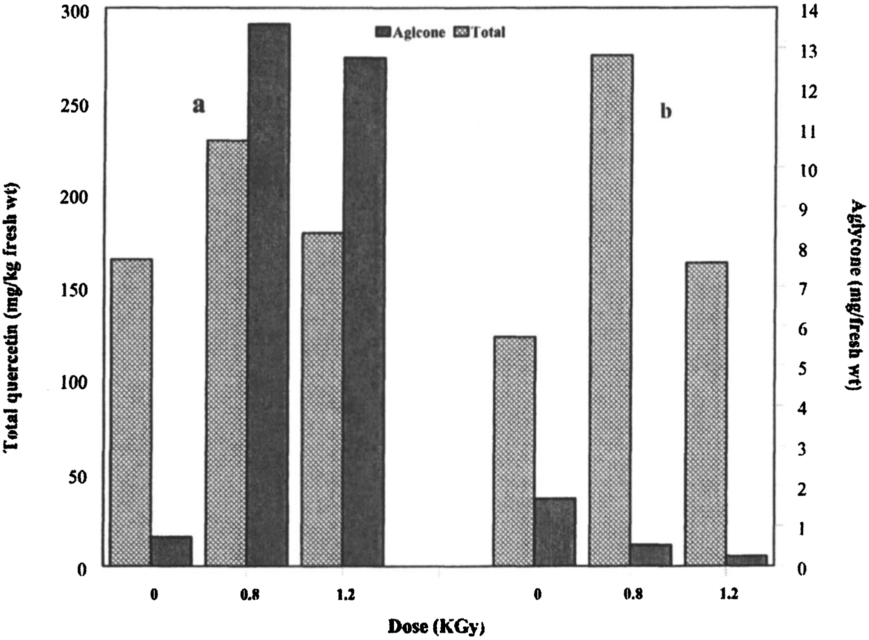

Figure 1. Gamma irradiation effect an aglycone and total quercetin content in 'Cardinal' (a)Diced onion and (b) Whole onion bulbs. (Adapted with permission from reference 79. Copyright 1999 Subtropical Plant Science.)

The loss of quercetin-3-glucoside (Q3G) was reported when glucose was added to the media by using rat jejunal (*81*). The Q3G is not absorbed when incubated with proximal colon tissue since there is no SGLT1 expression in the colon. Furthermore, it was demonstrated that quercetin 4'-β glycoside is absorbed via SGLT1 in Caco-2 cells, a model of intestinal absorption (*82*).

In general, gamma irradiation treatment of diced onions increased total quercetin content in yellow and red colored cultivars. Onions exposed to 0.8 kGy had the higher total quercetin content. The 1.2 kGy treatment also resulted in a slight increase in total quercetin content (Table I). An increase in the total quercetin content after irradiation treatment may be due to stimulation of PAL (Phenylalanine ammonia lyase) and flavonoid biosynthesis (*83*). Hahlbrock and Grisebach (*84*) reported that PAL is the rate-limiting enzyme in flavonoid glycoside biosynthesis in response to irradiation.

Flavonones and Flavones in Citrus

Flavonoids from different subgroups have been identified in citrus. The most abundant flavonoids in citrus differ with each species. For example, grapefruits contain flavonone (Figure 2c) and naringin (Figure 2d); oranges contain hesperedin (Figure 3a); mandrins contain polymethoxy flavones tangeretin (Figure 3b) and nobeletin (Figure 3c). One minor flavonone, narirutin (Figure 2e), is present in all three species. Other minor flavonoids in grapefruit are the flavonol (Figure 2a) glycoside quercetin and furanocoumarins (*61, 85*).

In general, most flavonones are pigments but citrus flavonones are colorless. The main difference between flavonones and other higher oxidized flavonoids is the optical activity shown by the flavonones due to the asymmetry center at C2. It must be noted that in nature only the levorotatory (-) forms with 2S-configurations exist. This flavonones can be converted into flavones and other flavonoids by specific enzymes. Another characteristic of these flavonoids is their ability to influence the taste of the plant in which they occur (*60*), as with the case of naringin. The bitterness of grapefruit is due to the presence of naringin and limonin, a triterpene. To reduce the bitterness of grapefruit, a hydrolytic enzyme called naringinase was added to break down naringin into naringenin, a non-bitter compound. The amount of naringin found in grapefruits varies between 806-1459 µg/g (*86, 87*).

Different stresses (irradiation, wounding, nutrient deficiencies, herbicide treatment, and viral, fungi, and insect attacks) have been shown to enhance either PAL synthesis or activity in different plants (*88*). Accumulative evidences have shown that irradiation influences of phenolic compounds (*89*) as phenolic biosynthesis is a distinctive response of plant tissue to abiotic stress and irradiation. Contrary to the previous study, several studies did not show accumulation of phenolic compounds after the peak of PAL activity (*90, 91*). Irradiation treatment, an oxidative stress, in grapefruit was induced (*91, 92*) and transient induction of PAL 24 h after treatment was noticed. Previously, PAL has been indicative of rate-controlling enzyme in phenolic synthesis and wounding of citrus. It has been reported that levels of phenolic compounds in irradiated grapefruit may be dependent on maturity (*93*). Studies in *Citrus clementina* showed a significant increase in polymethoxylated flavones (nobiletin and heptamethoxyflavone), flavonone, and hesperidin after 14 days of storage (*94*). In Mango, flavonoids and phenolic acids were enhanced due to irradiation (*95*).

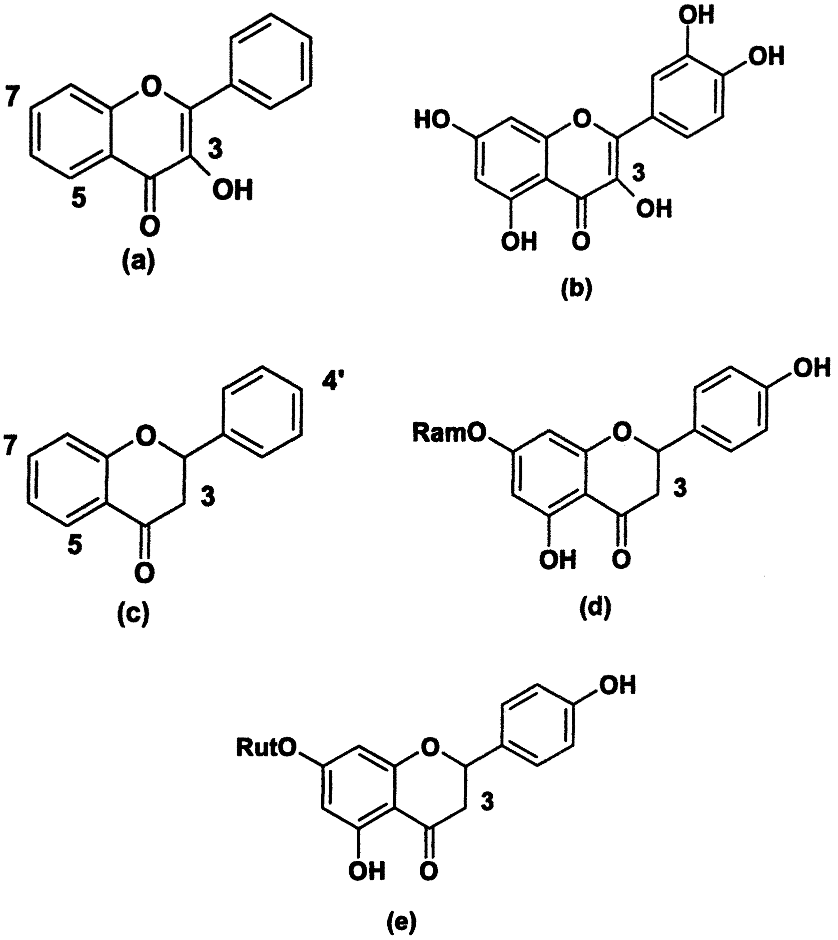

Figure 2. Structure of flavonoids, (a) Flavonol, (b) Quercetin, (c) Flavonone, (d) Naringin, (e) Narirutin.

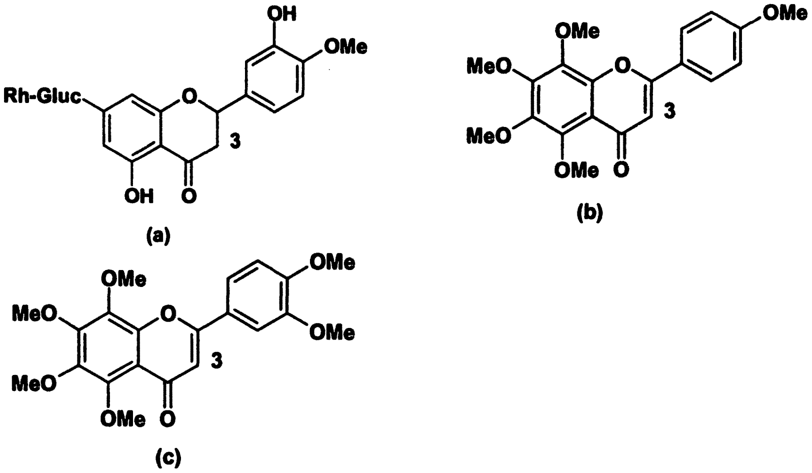

Figure 3. Structure of flavonoids, (a) Hesperidin, (b) Tangeritin, (c) Nobiletin.

Recently, our lab conducted a study to determine different levels of functional components in grapefruit due to irradiation. We found that gamma irradiation had a differential effect on early and late season grapefruit. Fruits were stored under simulated marketing conditions (4 weeks at 10°C and one week at 24°C under 90-95% relative humidity). In early season study, total flavonones (naringin and narirutin) concentration was significantly ($P \leq 0.05$) higher in fruits exposed to 200 Gy irradiation than any other treatments (0, 70, 400, and 700 Gy). Interestingly, both naringin and narirutin levels decreased as the irradiation dose increased (Figure 4). Oufedjikh and others also reported that the concentration of flavonone glucosides and polymethoxylated flavones were significantly lower in irradiated fruit (300 Gy) at 0 day of storage (*96*). The decline in flavonoids content of grapefruit explains their role in counteracting the oxidative stress induced by the gamma irradiation. Variations in the flavonone content at different doses of irradiation treatment may be a result of equilibrium between gamma irradiation induced oxidative stress and *de novo* synthesis of flavonoids by increased PAL activity. In late season harvested fruits, total flavonone content was significantly higher in non-radiated fruits than in irradiated fruit after the marketing simulation. Irradiation had no significant ($P \geq 0.05$) effect on naringin content of late season grapefruit. In general, total flavonone concentration decreased with increased irradiation dose even in the late season grapefruit, and storage had a positive effect on the flavonone concentration.

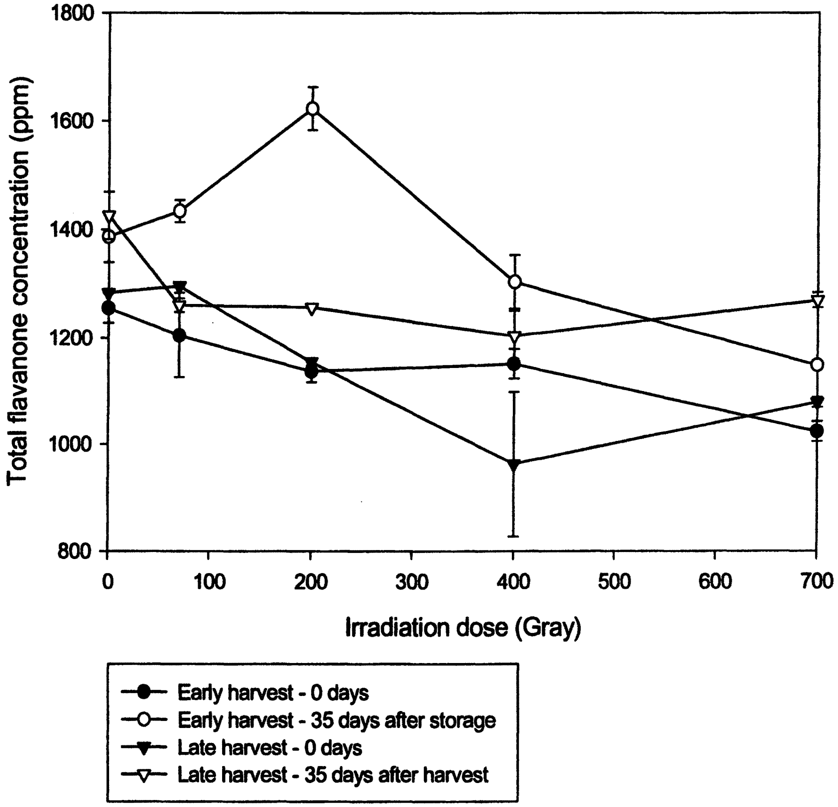

Figure 4. Irradiation and storage effects on total flavonone content of early and late season 'Rio Red' grapefruits.

Pectin

While accumulative evidence related to irradiation dose and security against quarantine treatments exists, studies related to irradiation and biochemicals changes in related compounds are yet to be ascertained. In recent years, changes in biochemical components due to irradiation have been established. Changes in pectic substances due to irradiation have been reported in different fruits such as apple, pear, peach, and mango (*97, 98, 99, 100*). Studies have confirmed that softening of papaya is retarded by irradiation (*101*), and irradiation doses lower than that recommended by the FDA for fruits and vegetables (≤1.0 kGy) (*15*) reduced respiration (*95*). In order to understand the effect of irradiation on the changes in pectin properties, studies conducted by Zhao and his colleagues revealed that a significant linear relationship exists between irradiation dose (0.25, 0.5, 0.75, 1.00 or 1.5 kGy) and firmness of papaya immediately after irradiation treatment (*102*). While irradiation increased water-soluble pectin, chelator soluble pectin and alkali soluble pectin decreased. In our recent studies (*103*), pectin significantly inhibited the binding of Fibroblast Growth Factor (FGF-1) to the FGF receptor (FGFR1) in the presence of 0.1 μg/mL heparin. The FGF-FGFR inhibition activity significantly correlated with sugar content, methoxyl content, and size of pectin. In addition, the lower relative ratio of rhamnose in pectin seems to be related with the higher inhibitory activity. This may be due to the fact that rhamnose plays a critical role in defining the three-dimensional structure of pectin (*104*), and therefore, enhancing its biological activity. It is important to understand the role of irradiation on pectin composition and its structural changes of individual sugar molecules within pectin for the potential health benefits.

Vitamin C

The major sources of vitamin C in the human diet are fruits and vegetables. Among the several postharvest factors that influence vitamin C, irradiation effects on tropical fruit and vegetable crops have been studied. Until recently, most available information in literature information is related to the influence of irradiation on ascorbic acid. Ascorbic acid (AA) is present in reduced form, while L-dehydroascorbic acid (DHA) is an oxidative product (*105, 106*). Since both DHA and AA are biologically active and irradiation has shown to oxidize AA to DHA, it is important to measure total vitamin C (AA plus DHA). Lower irradiation dose (300 Gy) does not appear to have significant effect on AA and DHA. Interestingly, irradiation could reduce losses in AA in potato compared to the control after potatoes were subsequently stored at 15°C (*107*). Higher doses (2-3 kGy) of irradiation in combination with refrigeration increased AA and decreased DHA levels in strawberries (*108*). In general, irradiation dose required for quarantine treatment for citrus did not show significant loss of vitamin C. In Spain, clementine fruits irradiated at 0.3 and 0.5 kGy doses along with hot water treatment (53°C, 5 min) and stored for 3 weeks at 17°C were reported to have higher vitamin C contents compared to the control (*109*). In grapefruit, higher irradiation dose of 1.5 kGy decreased vitamin C; however 0.25 kGy did not decrease vitamin C content (*110*). In our recent study, early season grapefruit irradiated up to 700 Gy did not have a significant effect on vitamin C concentrations. However, late season fruit exposed to a dose of irradiation greater than or equal to 200 Gy caused a significant reduction in vitamin C content after simulated marketing conditions. These results indicate that stress induced by irradiation above 200 Gy, coupled with low temperature stress, is detrimental to the late season crop.

Carotenoids

For about 50 years the nutritional and health interest in carotenoids was limited to provitamin A compounds. The ability to exert provitamin A activity is one of the important properties limited to those carotenoids with an unsubstituted β-ionone ring structure. The other important property of carotenoids is antioxidant activity. Carotenoids are efficient scavengers of singlet oxygen and free radicals (*111-113*). Several extensive reviews about carotenoids and cancer prevention have shown many properties of each carotenoid against a variety of cancers (*114-116*). Studies conducted with higher doses of irradiation treatment of fruits and vegetables indicated changes in vitamin content. Mangoes irradiated up to 2000 Gy showed a 10% increase in carotene content (*117*); however, carotene content of carrot was not affected by irradiation at 800 Gy dose (*118*). A very high dose of irradiation (18.6 kGy) reported a moderate loss of carotonoids in carrot (*119*). Apparently, these high doses of irradiation are not recommended for fruits and vegetables by the USDA and most of the disinfestation treatments require less than 300 Gy to sterilize several insect species (*4*).

Recent studies showed that lower doses (75 and 300 Gy) of gamma irradiation had no significant effects on carotene levels in mango and red capsicums. Modified irradiation conditions such as lower temperature (near 5°C), nitrogen atmosphere, and lower dose rate increased carotene concentrations compared to normal ambient conditions. Irradiated potato tubers stored at 15°C for seven months and reconditioning at 34 - 35°C for 6-12 days had a significant increase of 2-6 fold of carotene (*120*). Studies indicated that higher levels of carotenoids in potato tubers stored at 4 and 25-30°C compared to 15°C and 20°C could be explained due to the strong relation between temperature and synthesis and accumulation of carotenoids. Irradiation dose (100 Gy) used for potato sprout inhibition reduced lypoxygenase activity and appears that very little correlation exists between disappearance of carotenoids and enzyme activity (*9*).

Our studies illustrated that the effect of storage was more important than the effect of irradiation. In general, early season grapefruit had significantly ($P \leq 0.05$) higher levels of beta-carotene after the marketing simulation than late season. Thomas and Janave studied the carotenoid changes in irradiated mature mangoes stored at low temperature and found that low temperature storage resulted in increased carotenoid content. Irradiation had no effect ($P \leq 0.05$) on the beta-carotene content of grapefruit before or after the marketing simulation (*121*). Sebastiao et al. (*122*) reported that gamma irradiation doses of 0, 10 and 20 kGy did not affect beta-carotene content, nor did it contribute to the decrease of vitamin A in parsley. However, late season fruit treated with low doses of irradiation (70 Gy) had significantly ($P \leq 0.05$) higher levels of lycopene compared to fruits exposed to higher doses of irradiation (700 Gy) after irradiation and storage. In general, late season fruit storage has resulted in a decline in lycopene content. It is interesting to note that no differences ($P \leq 0.05$) were recorded between initial and final total carotenoid content in early season grapefruits.

Quality

Despite the potential benefits of irradiation, as one of alternative quarantine treatments, it is essential that fruit and/or vegetable products from these treatments are not adversely affected. It has been reported that peel injury in grapefruit may occur at an absorbed dose of 0.3 kGy (*17, 18*). However, harvest season, maturity of peel tissue at harvest, location of fruit (interior vs. canopy) may change the tolerance level (*123, 124*). Since grapefruit has a low tolerance limit to irradiation, it is important to determine specific pretreatments to reduce potential of irradiation damage. Among the possible preharvest treatments, gibberellic acid (*18*), and postharvest pretreatments such as vapor heat (VH) or fungicides (*125*), as well as heat conditioning (*123*) were attempted. Both preharvest gibberellic acid (GA) and postharvest (VH and heat conditioning) treatment significantly reduced the incidence and severity of pitting caused by irradiation depending on the dose level. Vapor heat and heat conditioning treatment effect could be ascribed to accumulation of heat shock proteins (*126*). In GA-treated experiment, no significant changes in total soluble solids (TSS) and titratable acidity (TA) were reported due to GA or irradiation (*18*). Total soluble solids and TA percentages were lower in fruits exposed to VH compared to control fruit and these values decreased as irradiation dose increased from 0 to 1.0 kGy (*125*). Similar decreases in TSS and TA were observed in heat conditioning experiments as the irradiation dose increased from 0 to 1.0 kGy. Heat conditioning from 38 to 42°C increased mean ratio of TSS:TA (*123*). Studies conducted in our laboratory demonstrated no adverse effects on TSS, acidity or brix/acid ratios due to irradiation or storage of early season fruit. These results are in agreement with the results of Moshonas and Shaw (*125*). However, late season fruit had lower TSS and acidity values than early season fruit and the brix/acid ratio after storage at simulated marketing conditions and the ratios were slightly higher than the initial ratios. Irradiated late season fruit retained acidity better than control. Initial TSS in fruits was lowest ($P \geq 0.05$) in the late season fruits exposed to 700 Gy irradiation, however no differences between treatments were observed after storage.

Sensory Evaluation

In general, lower irradiation doses (150 and 300 Gy) induced certain changes in grapefruit flavor that was preferred by taste panel judges compared to higher doses (600 and 900 Gy). Adverse flavor changes were reported in fruits from early season irradiated fruits, and flavor rating was improved in the fruit harvested from December through May (*127*). The relationship between soluble pectin and flavor has been yet to be established. Grapefruit trees sprayed with gibberellic acid (GA) and lower dose (0.3 kGy) of irradiation applied to grapefruit did not affect juice flavor, pulp flavor and texture compared to control. However, a higher irradiation dose (0.6 kGy) resulted in a lower preference rating of irradiated fruits compared to control (*18*). Vapor heat had a slight positive influence on juice flavor, and a slight negative influence on pulp flavor and pulp texture when the preference ratings of all doses of irradiation (0.0, 0.5, and 1.0 kGy) were averaged. Furthermore, similar to GA-treatment experiment, the preference rating in vapor-heat and heat-conditioning juice flavor, pulp flavor and pulp texture declined as irradiation dose increased (*123, 125*). Rio Red grapefruit's sensory evaluation conducted in our lab using untrained panelists demonstrated that appearance and flavor of early season grapefruit exposed to irradiation treatments at or below 200 Gy were comparable to the control after the simulated marketing conditions with the exception of the 700 Gy treatment, which was found to be detrimental. Appearance of grapefruit was more susceptible to irradiation than flavor. Irradiation had no significant ($P \leq 0.05$) effect on the sensory qualities of late season grapefruit. Nunez-Selles and co-workers (*128*) reported that organoleptic evaluation of juice from irradiated grapefruit (750 Gy) showed that the effect of the treatment was not greater than that of storage.

In California oranges, trained panelists were able to detect the changes in appearance of whole fruit, and in flavor, taste, and odor of juice when fruits were irradiated below 0.6 kGy dose as compared to control (*129, 130*). Sensory panelists in Brazil found that tangerines treated with irradiation up to 1.0 kGy dose was acceptable, but those treated with a combination of hot water followed by irradiation were not acceptable (*131*). Another study in Spain with clementine showed similar results (*109*).

This chapter is focused on the effect of irradiation on functional components and specifically discussed the irradiation effects at doses required for quarantine purposes in grapefruit. Extensive literature of irradiation effect on nutritional components in tropical and subtropical as well as temperate fruits and vegetables is published by Thomas (*132-135*). Currently, irradiation is used as a quarantine treatment for Mexican fruit fly; it is our hope that the positive and negative benefits of irradiation on potential health properties of citrus are taken into consideration while using the treatment.

Acknowledgments

Funding for this research was provided through a grant from USDA-CSRRES (2000-34402-9589). The author would like to thank Dr. Guy Hallman and Dr. John Jifon for their critical comments during the manuscript preparation.

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Chapter 9

Low-Dose Ionizing Radiation of Fruit Juices: Benefits and Concerns

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Ionizing radiation of fruit juice has been studied for a half century. Low dose radiation effectively inactivates foodborne pathogens, and reduces patulin (a mycotoxin) and brownness. However, irradiation induces undesirable chemical changes, such as accumulations of malondialdehyde, formaldehyde, and tetrahydrofuran. Published literature on negative flavor change of juice due to low dose radiation is contradictory. Evidence exists concerning the involvement of volatile sulfur compounds in the development of off-flavor. Many of the undesirable effects of irradiation can be reduced by conducting irradiation at low temperature, by addition of antioxidants, and by combining irradiation with other techniques and treatments, such as mild heating and use of antimicrobials.

U.S. government work. Published 2004 American Chemical Society

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Ionizing radiation is a non-thermal processing technology that has been extensively studied for preservation of foods including fruit juice. Most early researchers, using high doses (more than 10 kGy), mainly focused on sterilization of juice that created shelf-stable products at ambient temperature. Off-flavor and loss of ascorbic acid (AA) are often encountered at those high doses. Recent research has focused on inactivation of foodborne human pathogens and improvement of food safety using lower doses (less than 5 kGy). The present paper reviews the beneficial and the adverse effects of ionizing radiation on fruit juice, and discusses means to minimize the undesirable effects induced by irradiation. Original research is also presented.

Beneficial Effects of Ionizing Radiation

Human Pathogen Inactivation

In the United States, foodborne infections caused an estimated 76 million cases of illness (16,000-148,000 cases attributed to juice), 325,000 hospitalized and 5,000 deaths every year (*1*). Serious incidences of salmonellosis and outbreaks of *E. coli* O157:H7 are associated with consumption of orange juice and apple juice/cider (*2, 3*). Un-pasteurized juice may account for 76% of juice contamination cases reported between 1993-1996 (*4*). The foodborne infections involving fresh juice have increased consumer awareness of food contamination with pathogens. To address the problem, FDA has ruled that any fruit or vegetable juice processor must utilize processing technologies and HACCP programs to achieve a 5-log reduction of the most resistant pathogenic microorganisms (*5*). Under the rule, juice and juice products that have not been specifically processed to attain a 5-log reduction in the pertinent pathogen must bear a warning label. Most processors use thermal processes to achieve the 5-log reduction. However, thermal pasteurization damages the fresh flavor and aroma of juice. Therefore, alternatives other than thermal processing have been explored. Ionizing irradiation has been shown to effectively inactivate human pathogens in fruit juice. Buchanan and others (*6*) studied inactivation of *E. coli* O157:H7 in apple juice by irradiation. They found that the acid adaptation of bacteria affected the radiation resistance of the pathogen. Radiation resistance is commonly expressed as D_{10} values, which are radiation doses required to inactivate 90% bacterial population. Non-acid-adapted *E. coli* O157:H7 was more radiation sensitive with the D_{10} values ranging from 0.12-0.21 kGy than acid-adapted cells with D_{10} values of 0.22-0.31 kGy. The authors concluded that a dose of 1.8 kGy should be sufficient to achieve the 5-D inactivation of the bacterium. Niemira and others (*7*) found the D_{10} values for Salmonella varied among species and among isolates. *S. anatum* (D_{10} = 0.71 kGy) was significantly more resistant than the other serotypes tested. *S. newport* (D_{10} = 0.48 kGy) and *S. infantis* (D_{10} = 0.35 kGy) differed significantly from each other, while *S.*

stanley (D_{10} = 0.38 kGy) was intermediate between the two. Salmonella sensitivity to ionizing radiation was not strongly influenced by juice composition with regard to antioxidants, calcium, or suspended solids of formulated commercial orange juice (*8*). However, amendments such as vitamin A and E had a more pronounced effect on *Salmonella* radiation sensitivity in orange juice (B. A. Niemira, unpublished). pH had a significant effect on radiation resistance of pathogens, lower pH generally increased radiation sensitivity of bacteria (*9*). A 5-log reduction in *Salmonella hartford* and *Listeria monocytogenes* can be achieved in fresh orange juice by treatments with 2.65 and 2.4 kGy gamma radiation, respectively. From the above studies, we can conclude that a dose of 3.5 kGy is sufficient to achieve a 5-log reduction of the most resistance pathogen (*S. anatum*) required by the FDA.

Destruction of Patulin

Patulin is a mycotoxin produced by several fungi commonly grown on apple fruit, particularly if fruit are bruised or damaged. Patulin has been reported to be mutagenic and to cause neurotoxic, immunotoxic, genotoxic, and gastrointestinal effects in rodents (*10, 11*), although little evidence exists that patulin is carcinogenic to humans. The long-term toxic effects of patulin in young children are of particular concern because children consume large amounts of juice relative to body weight. In 2001, the FDA set the maximum limit of patulin to be 50µg/kg in apple juice and single strength apple juice from concentrate (*5*). Proper fruit selection, handling, sorting, and washing can assure good quality fruit is used to make apple juice, and therefore meet FDA requirement. While product is still rejected due to high levels of patulin, the rejection rate has decreased in recent years (5).

Ionizing radiation can be used to destroy patulin (*12-14*). As radiation doses increased, patulin content of apple juice decreased sharply. The dose which reduced the patulin content by 50% of its initial values was only 0.35 kGy in apple juice concentrate (*12*). Therefore, low dose radiation can be used to reduce patulin in fruit juice to meet the requirement of patulin limit set by FDA and international trading and regulatory agencies.

Color Changes

Fan and Thayer (*15*) found that gamma radiation significantly reduced brownness of apple juice measured as absorbance at 420 nm. Even at 1.0 kGy, the brownness of apple juice measured as absorbance at 420 nm was reduced by 60.5 %. After 15 days storage at 5 °C, the absorbance of juice was still much lower than that of non-irradiated juice (*15*). Reduced brownness by irradiation was also observed in apple juice concentrate, though to a less extent (*13*), presumably due to presence of higher amount of antioxidants and other compounds in the juice concentrate. Figure 1 shows the changes in the absorbance of non-irradiated orange juice and those irradiated with doses of

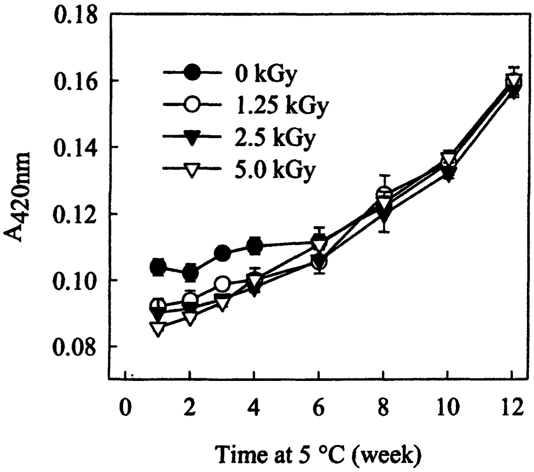

Figure 1. Change in browning of orange juice during storage. Single strength orange juice irradiated at 0, 1.25, 2.5, and 5 kGy was stored at 5 °C. Brownness was measured as absorbance at 420 nm after dilution (1:1) with ethanol and centrifuge at 12,000 g for 10 min at 5 °C.

1.25, 2.5 and 5 kGy during 12 weeks of storage at 5 °C. Although the brownness (measured as absorbance at 420 nm) of irradiated orange was lower than non-irradiated samples immediately after irradiation and within 4 weeks storage, the brownness of all samples became similar after 6 weeks of storage, indicating that the rate of non-enzymatic browning during storage was higher for irradiated juice than non-irradiated juice. However, Zegota and others (*13*) found that the rates of browning in irradiated apple juice concentrate are similar as those of non-irradiated samples during 16 weeks of storage at 4 °C. Color of apple juice concentrate irradiated with the dose of 2 kGy was preferred by consumers compared to non-irradiated samples (*14*). The loss of brown color caused by irradiation may be due to the degradation and bleaching of browning pigments. Chachin and Ogata (*16*) found that anthocyanin in grape juice was sensitive to gamma radiation but β-carotene was stable in orange juice.

Other beneficial effects of juice irradiation include inactivation of enzymes (*17, 18*), increase in antioxidant activity (*15*) and filtration rate (*19*), and reduction in fermentation (*20*). To achieve significant changes in enzyme activity, high doses are generally required.

Fan and Thayer (*15*) have showed that antioxidant capacity of apple juice, measured by the ferric reducing antioxidant power (FRAP), increased at doses above 0.9 kGy immediately after irradiation. However, the increased FRAP values disappeared during storage at 5 °C.

Fresh juice tends to ferment during storage. Irradiation alone or in combination with mild heating can be used to increase the shelf life by inactivating the yeast responsible for fermentation. Hussain and Maxie (*20*) studied the shelf life of orange juice inoculated with *Saccharomyces cerevisiae* var. ellipsoideus. They found that untreated orange juice cannot be stored at room temperature for greater than 4 days; heated samples (50 °C for 20 min) can be stored for less than 6 days. Heat treatment after irradiation (1-5 kGy) resulted in longer storage life (16 days) compared to heat treatment before irradiation (12 days). Effective pasteurization of orange juice with acceptable quality was achieved by a dose of 3 kGy gamma rays followed by heating for 20 min at 50 °C.

Irradiation of fruit juice at low doses did not change the content of reducing sugars, organic acids, phenolics compounds, or amino acids (*13, 16*).

Adverse Effects of Ionizing Radiation

Loss of Ascorbic acid (AA)

Juice is a major source of AA in the American' diet (*21*). Loss in AA is not only important nutritionally, but also relates to flavor and color changes in juice (*22*). AA has been found to be sensitive to irradiation (*23*). Upon irradiation, AA is converted to dehydroascorbic acid (DHA) under aerobic condition, and DHA undergoes further degradation because DHA is less stable compared to AA. Both AA and DHA are similar in terms of nutrient value (antiscorbutic property). Many studies, however, have measured only AA, not DHA. Zegota et al. (*12, 13*) found there was less than 4% of AA loss per each kGy in apple juice concentrate. Fan and others (*24*) showed the loss of AA in single strength orange juice was 6.9% per kGy while loss of total AA (AA plus DHA) was only 2.7% per kGy (*24*). Nyambati and Langerak (*25*) showed AA content of lime fruit pulp was reduced by 16% by 3 kGy radiation. Irradiation induced degradation of AA in the presence of air was affected by the concentration of AA (*26*). The amount of AA loss increased with higher AA concentration, but the percentage of AA loss decreased with higher AA concentration.

Degradation products of AA may include malondialdehyde (MDA), acetaldehyde and formaldehyde (FA) (*27*). Formation of acetaldehyde from AA upon UV radiation has been proposed (*28*). Other degradation products of AA are oxalic acid, keto-threonic acid, reductone A, reductic acid, glycolic acid and glyceric acid as detected by paper chromatography (*26*).

MDA and FA Accumulation

Malondialdehyde (MDA) is mutagenic in *Salmonella* test stains (*29, 30*), and initiates skin tumors in mice (*31*). High dose radiation induces the accumulation of MDA in aqueous carbohydrate solutions (*32, 33*). Irradiated carbohydrate solutions are toxic to mammalian cells *in vitro* (*34*), but has no genetic damage to *Drosophila melanogaster* (*35*). Lee and others (*36*) found vegetable juices irradiated to 10 kGy gamma radiation had no mutagenic effects in *E. coli* PG37 or cultured Chinese hamster lung fibroblast cells. Many earlier studies, using non-specific methods with strong acidity and elevated temperatures conditions may seriously overestimate the amount of MDA. Using a non-specific method, Fan and Thayer (*15*) measured MDA in orange juice as affected by irradiation. They found the levels of MDA equivalents were increased only at doses of 2.7 kGy and above. Later, a new method based on chemical derivatization and GC-MS separation/identification was developed, which was much more accurate and sensitive than traditional methods (*37*). Formation of MDA increased linearly with radiation dose at a rate of 62 ng/g per kGy (*27, 37*). The G-value (number of species formed per 100 ev absorbed) for formation of MDA was 0.0086 in fresh apple juice. The accumulated MDA, however, decreased dramatically during storage at 5 °C. Within 3 days, about half of MDA disappeared.

MDA is universally present in a variety of foods. MDA is best known to be formed from auto-oxidation of unsaturated fatty acids. MDA equivalent has been used as an index of rancidity and oxidative deterioration in meats. Bergamo and others (*38*) using a HPLC method found that meat samples contained 32-164 ng/g of MDA. Considering the rapid post-irradiation decrease in MDA levels in juice, the levels of MDA in juice irradiated for the control of foodborne pathogens are comparable to those commonly found in meats.

Ingestion of FA in drinking water can cause chronic oral toxicity in rats (*39*). U. S. Environmental Protection Agency classifies FA as B1 (probable human carcinogen). Irradiation increased the formation of FA in apple juice at a rate of 0.23 μg/ml per kGy (*27*). At 3.5 kGy, apple juice can accumulate 912 μg/L of FA, a level lower than the EPA health advisory level (1000 μg/L) (*40*).

Other Compounds

Information about toxicity of tetrahydrofuran (THF) is limited. Chhabra and others (*41*) showed that rats and mice developed elevated carcinoma if animals were exposed by inhalation at high concentrations (1800 and 5000 ppm). Developmental effects are only observed at exposure levels producing other toxic effects in adult animals. Bacterial and mammalian cell culture studies demonstrate no mutagenic activity with THF. Using a titrimetric method, Herrmann et al (*42*) found that THF concentration increased linearly with increasing radiation dose. At 3.5 kGy, apple juice can accumulate up to 21 mg/L of THF calculated from the linear response (*42*). Several U. S. states have maximum levels for THF in drinking water. For example, Massachusetts has a maximum contamination level of 1.3 mg/L of THF in drinking water (*43*). Other compounds possibly accumulated in irradiated fruit juice include glyoxal (*44*), hydroxymethylfurfural (*44*), glucosone (*45*) and methoxyacetaldehyde (*46*). Many of compounds listed above were identified in irradiated carbohydrate solutions. The accumulation of the compounds in fruit juice will probably be minimal or significantly reduced (*45, 46*). It should also be pointed out that most of the compounds including THF were identified and quantified using non-specific methods, which may result in over-estimation.

Flavor Changes

Spoto and others (*47*) irradiated orange juice concentrate at 0, 2.5, 5.0 and 7.5 kGy and stored the samples at 0, 5, and 25 °C for 1, 30, 60 and 90 days. Sensory attributes were evaluated by a trained panel using quantitatively descriptive analysis. They found juice concentrate irradiated at doses above 5 kGy developed an off-flavor with higher ratings in 'medicinal', 'bitterness' and 'cooked' attributes. The effect of radiation on flavor and aroma attributes depends on dose, storage temperature and time. They concluded that 2.5 kGy in combination with 0 and 5 °C of storage conditions provided a feasible approach for preserving juice concentrate. Thakar et al. (*48*) found irradiated (10 kGy) orange juice is less acceptable than non-irradiated juice due to development of the off-flavor. Foley and others (*9*) showed that fresh orange juice irradiated at doses as low as 1 kGy can produce "plastic to decayed" flavor, and render it unpalatable. Apple juice had different response to irradiation. Irradiated apple juice was preferred by consumer panels compared to pasteurized juice (*49*).

Fetter and others (*50*) found that irradiation at 20 °C with doses under 5 kGy did not significantly affect taste of any of the 12 juices and nectars including apple and orange juices, except grape nectar. Taste deteriorated with doses greater than 5 kGy. The degree of deterioration varies greatly with the type of drink. Clear juices are influenced to a much greater extent than are those containing fruit pulp.

Zegota (*14*) found at doses of 0.5-1.5 kGy there were little differences between irradiated and unirradiated samples in odor and taste (*14*). Apple juice concentrate irradiated with the dose of 2.0 kGy had slightly different sensory properties than the unirradiated ones (*14*). Herrmann et al. (*51*) found apple juice concentrate (40-50%) can be irradiated at 10 kGy without detectable 'irradiated' flavor in the diluted product (12%).

Volatile Compounds

Gasco et al. (*52*) found that irradiation at high dose (up to 28.9 kGy) had higher concentration of acetone and isopentanal, and lower hexanal and 2-hexenal content. Spoto et al. (*47*) found that irradiation-induced 'bitter', 'medicinal' and 'cooked' flavor was associated with hexanal, octanol, terpinene-4-ol, cis-carvenol, nerol, carvona, geraniol, perilyl alcohol, and cariophilene (*53*). Removal of THF using mercury acetate eliminates the off-flavor, promoting Herrmann et al. (*51*) to suggest THF may be the key substance in irradiation flavor. However, THF has an ethereal odor and high odor threshold (2-50 ppm). It is therefore doubtful that the irradiation-induced odor is due to THF.

Fan and others (*54*) did not find any significant effect by irradiation on the majority of volatile compounds (mainly terpene compounds) in orange juice. However, acyclic monoterpenes such as geranial, neral, myrcere and linalool were reduced by irradiation. At 3.55 kGy, at which dose the number of *Salmonella* and *E. coli* O157:H7 would be reduced by at least 5 log, these compounds may be reduced by as high as 23%. Chachin et al. (*55*) found that gamma radiation (5-20 kGy) increased acetaldehyde, propylaldehyde and butylaldehyde content in apple pulp and propylaldehyde and acetone content in apple jam. Fan and Thayer (*27*) also found that irradiation of apple juice increased formation of formaldehyde and acetaldehyde. Boylston and others (*56*) found that there was no difference in flavor and aroma between irradiated apple cider and pasteurized apple cider. Irradiation treatment (2 kGy) resulted in a decrease in the content of esters characteristic of apple flavor and an increase in the content of alcohols and aldehydes formed through lipid oxidation.

It appears that there are contradictions on whether irradiation induces off-flavor in fruit juice. The type and composition of juice may affect the development of off-flavor. Recently, Yoo and others (*57*) found concentrations of methyl sulfide and dimethyl disulfide in orange juice increased with radiation dose. In contrast to orange juice, concentrations of methyl sulfide and dimethyl disulfide in irradiated samples were lower than non-irradiated ones (X. Fan, unpublished). These results suggest that different type of juice may have different responses to irradiation in term of volatile sulfur production. Many volatile sulfur compounds had very low odor threshold and pungent odor (*58*). It is possible volatile sulfur compounds together with the other compounds, such as aldehydes, may be involved in the off-flavor development.

Techniques for Minimizing the Undesirable Effects

Heating

The undesirable effects of irradiation are dose dependent. Therefore, irradiation can be used in combination with other pathogen-reduction techniques, so lower doses are employed. Pasteurization is traditionally used for destruction of pathogens in juice, although it diminishes the 'fresh' flavor. It is possible to combine "light' or "ultralight' pasteurization or mild heating with ionizing radiation to ensure 5-log reduction of pathogens while minimizing the impact on flavor. It has been shown that combining heat with radiation was effective to inactivate *Salmonella typhimurium* (*59, 60*). The greatest effect of temperature during irradiation occurred at temperatures above 43 °C. Rate of bacterial destruction was significantly greater when the ionizing radiation and heating are applied simultaneously than when they were applied consecutively. Others (*61, 62*) also found synergism between mild heating and radiation on the inactivation of other microorganisms. The heat may enhance the irradiation effect by inhibiting the enzymatic repair process (*61, 62*), and inducing membrane destabilization (*60*). Combination of heating (50 °C, 10 min) with irradiation at 4 kGy increased shelf life of pear and apple juice by totally inactivating the microbial population (*63*). Apple juice heated to 70 °C for 8 sec followed by 3.5 kGy radiation had excellent sensory scores after 4 weeks of storage at 30 °C (*64*).

Combined heat and radiation processing of orange juice does not reduce thiamine (a vitamin) content in a synergistic fashion (*65*).

Use of Low Processing Temperature

Ionizing radiation executes its effect mainly through free radicals (such as hydroxyl radicals) generated from radiolysis of water in non-frozen aqueous foods. Temperature has an influence on the generation and mobility of free radicals. As processing temperature goes down, the mobility and diffusion rate of radical decreased, resulting in less radiation effect. Lodge et al. (*66*) found that irradiating kiwifruit pulp at −18 °C at a dose of 1 kGy resulted in a 2-log reduction in aerobic plate count. Assessments carried out over 6 months of storage showed no significant difference in physical, chemical, and sensory properties between irradiated and non-irradiated pulps. However, it should be pointed out that radiation resistance of bacteria also increased with reducing processing temperature. The efficacy in reducing the non-desirable effects of irradiation must outweigh the reduction in beneficial effects. As processing temperature goes down from 0 °C to −20 °C, the increase in MDA is almost completely prevented (27), however, radiation resistance of pathogens increases only by 2-3 fold (*67*, *68*).

Addition of Antioxidants and Antimicrobials

Radiation-induced off-flavors and browning in orange juice were completely inhibited by addition of sorbic acid (0.1%) (*48*). The loss of AA was also reduced. Nisin or tylosin increased radiation sensitivity of *Clostridium pasteurianum* in tomato juice (*69*). Fan and Thayer (*27*) found addition of ascorbic acid, sodium sulfite and potassium sorbate reduced the radiation-induced MDA accumulation. Chachin and Ogata (*16*) found addition of propyl gallate and AA to orange juice reduced the deterioration of juice caused by high doses (5-20 kGy) of radiation.

The loss in AA can be reduced by addition of antioxidants, organic acids or supplementation. Umeda and others (*26*) showed that nitrogen flushing and addition of various organic acids such as maleic acid and oxalic acid are protective against loss of AA due to irradiation.

The amount of off-flavor and consequent loss of quality may be reduced in some irradiated foods if radiation was delivered at a very high dose rate (*70*). However, the high dose rates of radiation may also be less effective in reducing the viability of pathogens (*71*) depending on type of pathogen and foods. Other means of reducing the undesirable effects of irradiation may include exclusion of oxygen from juice sample and removal of undesirable volatiles (*72*).

In summary, ionizing radiation had many benefits in fruit juice, however there are concerns on the chemical safety and developments of off-flavor. All chemicals induced by low dose radiation appear to be natural occurring compounds. It is unclear whether the accumulation of these compounds causes any health problem. Contradictions exist on the development of off-flavor in fruit juice irradiated at low doses. The development of off-odor may depend on the type of juice, and production of volatile sulfur compounds.

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Chapter 10

Ionizing Radiation of Seafood

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Low dose ionizing irradiation has proven to be effective in reducing pathogenic and spoilage microorganisms in a variety of seafood products. Although irradiation processing has not yet been approved for use in seafood products in the United States of America, there has been extensive research conducted on molluscan shellfish, crustaceans, and finfish since the 1950's. Petitions have been submitted to the US-FDA Office of Food Additive Safety (previously the Office of Premarket Approval) for approval for irradiation processing of molluscan shellfish and crustaceans; a petition for finfish is being finalized.

Introduction

Irradiation of seafood can utilize ionizing radiation from a variety of sources. The sources that have been most effective in eliminating microorganisms, while maintaining the integrity and quality of seafood, are X-rays, gamma rays, or electron beam accelerators. These three sources have the capability of penetrating into, as well as through, most food products. When attempting to control microorganisms for microbial remediation or extension of shelf-life in fresh seafood products, the time of exposure and the specific irradiation dose are very important for maintaining the integrity and fresh like quality of the product. Over-processing can adversely affect the protein nature and consequently the sensory quality of the product. Most research studies, with seafood products, have encompassed both the microbial and sensory aspects of

(This writing was approved for publication by the Mississippi Agricultural and Forestry Experiment Station, MAFES # CC10336)

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

irradiation processing. The advantages of using low dose ionizing radiation (<3 kGy) as a processing method are twofold: first, it will reduce or eliminate 90-95% of the microorganisms responsible for spoilage and subsequently will extend the fresh-storage shelf-life; second, this same low dose of irradiation has the ability to reduce or eliminate specific pathogenic bacteria commonly associated with seafood (*1*). For example: fresh shrimp held on ice normally maintain good quality for up to 7 days, but by irradiating shrimp at low dosage (1.5 kGy) it is possible to extend the fresh quality for an additional 7-10 days. Grodner and Hinton (*2*) reported that the lethal dose of 1 kGy was effective in eliminating *Escherichia coli* and *Vibrio* spp in oysters. This level of irradiation did not change the appearance or "raw" quality of the oysters.

Historical Perspective

Early efforts to determine the feasibility of using irradiation processing for seafood products began with the Marine Products Development Irradiator (MPDI) in Gloucester, MA. This irradiator was built from funding by the Atomic Energy Commission (AEC) with the intent to be used as a development facility for the seafood industry. In the early to mid-1960s, several government laboratories of the Bureau of Commercial Fisheries (BCF), now evolved into the National Marine Fisheries Service (NMFS) began radiation research on a variety of seafood products. The laboratories included Gloucester in the Northeast, Ann Arbor for the Great Lakes region and Seattle for the Pacific Northwest. Several universities were also supported by AEC funding and included Louisiana State, Oregon State, California and Washington. Internationally, Canada's Halifax and Vancouver laboratories, Torry Research Station in the United Kingdom, and research centers in the Soviet Union, Japan and Australia all joined in this effort. "Much of the early research on seafood irradiation was on shelf-life extension (Table I), primarily involving sensory testing and microbiology" (*3*).

Table I summarizes the shelf-life data for several species of fish and shellfish. Optimum dose was that which provided the longest shelf-life without altering the normal sensory characteristics of the product. Maximum dose was the level where sensory characteristics began to show significant changes. In general, low fat species are less susceptible to sensory changes from irradiation processing. Very high fat species and those with intense color, including salmon, may show changes in color (bleaching) or undergo unacceptable lipid oxidation. Sterilization of fish products by irradiation processing requires 40-50 kGy dose levels and produces an unacceptable product. Early research by the various universities and in cooperation with the United States Army Natick Laboratories determined that radiation sterilization of seafood was not commercially feasible. Over the past 40 years, most of the research on seafood irradiation processing has been conducted by university research centers. A review of these various research activities follows.

Table I. Shelf Life and Optimum Dose Research Results Reported by NMFS Gloucester Laboratory

Species	*Optimum Dose (kGy)*	*Maximum Dose (kGy)*	*Iced Shelf Life (Days)*
Molluscan Shellfish			
Soft Shell Clam, *Mya arenaria*	4.5	-	30
Surf clam, *Spisula solidissima*	4.5	-	40
Oysters (species not given)	2.0	8.0	21
Crustecean			
Shrimp	1.5-2.0	5.0	21-30[3]
Finfish			
Monkfish, *Lophium americanus*	1.5	-	20
Butterfish, *Peprilus triancanthus*	2.3	4.6	49
Cod, *Gadus morhua*	1.5	4.6	30
Dogfish, *Squalus acanthias*	2.0	2.0	7[4]
Winter Flounder, *Pleuronectes americanus*	4.5	9.3	22
English sole, *Parophus vetulus*	2-3	-	28-35[1]
Gray sole, *Glyptocephalus cynoglossus*	1-2	-	29
Atlantic Halibut, *Hippoglossus hippoglossus*	2-3	5.0	30[2]
Petrale sole, *Eopsetta jordani*	2-3	3.0	28-49
Yellowtail flounder, *Limanda ferruginea*	1-2	-	21-25[1]
Haddock, *Melanogrammus aeglefinus*	1.5-2.5	6.7	30-35
Herring smelt, *Argentina silus*	0.5-1.0	-	15
Mackerel, *Scomber scombrus*	2.5	-	30-35[2]
Ocean perch, *Sebastes marinus*	1.5-2.5	-	30[2]
Pollock, *Pollachius virens*	1.5	-	28-30
Whiting, *Merluccius billincaris*	1.2	2.0-2.5	24-28

[1] In cooperation with NMFS Seattle Laboratory

[2] Vacuum-packed

[3] In cooperation with Louisiana State University

[4] Became rancid after seven days on ice

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Irradiation Effect on Microorganisms in Seafood

Ionizing radiation inflicts damage to large macromolecules including nucleic acids. Since the life and reproduction of bacteria are dependent on their nucleic acids, most microorganisms are destroyed. Species of microorganisms exhibit differing resistance to ionizing radiation. Sensitivity variations may even occur among strains of the same species. Gram-negative bacteria are generally considered more sensitive than gram-positive species; consequently, many of the typical spoilage bacteria are among the least resistant. Each species of bacteria, as well as the particular seafood substrate with which one is concerned, should be examined on an individual basis. For example, there is a great variation among the different *Salmonella* seros in different seafood. Gram-positive bacteria such as *Staphylococcus aureus, Micrococcus, Bacillus*, and *Clostridium* are among the more irradiation-resistant genera. Viruses, in general, are extremely resistant to irradiation at the low dosage levels applied to seafood products. Fish parasites also require a fairly high dosage to be inactivated.

Spoilage Microorganisms

The early research work in seafood irradiation processing primarily focused on extension of shelf-life. Fresh fish and shellfish are highly perishable products with limited shelf-life, especially when you consider shipping to interior areas away from coastal waters. Shelf-life extension of even a few days increases the viability of the industry and marketability of seafood products. Seafood quality is determined by subjective sensory judgment of the consumer. In addition, microbiologists often use levels of aerobic spoilage bacteria (Aerobic Plate Counts) as an objective measure of freshness. This method gives a good measure of product sensory quality and whether the product could pose a food safety problem. The microbiological flora of freshly caught fish and shellfish naturally reflects that of their harvest environment. The predominant bacterial floras of freshly caught fish or shellfish are the gram-negative *Pseudomonas* groups, which often compose 60% of the total flora (*4*). Other microbial species implicated in spoilage of fresh-caught marine fish include *Micrococcus, Flavobacterium*, and *Crytophaga* with *Corynebacterium*, *Vibrio*, *Bacillus, Proteus*, and yeasts (*5*).

Shelf life can be increased by at least 7 days for most species of fish and shellfish using ≤2 kGy irradiation processing. At this level, spoilage microorganisms are reduced by 99.9%. A summary documenting these results follows (*4, 6*).

- Molluscan shellfish
    - Shucked oyster meats, irradiated at 2.0 kGy and stored on ice, demonstrated a shelf life of 21-28 days compared to 15 days for unprocessed controls (*7, 8, 9, 10*).
    - Liuzzo et al. (*11*), using 2.5 kGy, obtained a 7 day shelf life extension for shucked oyster meats.
    - Irradiation of shell stock oysters has received mixed review: Mallett et al. (*12*) obtained a 25 day shelf life using < 2.5 kGy. Other researchers have found that although spoilage bacteria are reduced, the survival rate for live oysters was also reduced at levels greater than 1 kGy. General consensus is that at low doses (1.0-1.5 kGy) shelf life based on oyster survival is similar to unprocessed oysters (*13,14,15*).
    - Air-packaged shucked scallop meats, irradiated at 0.75 kGy, achieved a 2-3 week shelf life extension beyond unprocessed control (*16*).

  - Shucked surf clams, irradiated at 0.45 kGy, demonstrated a 30-40 day shelf life extension beyond unprocessed control (*17*).
  - Both dried and roasted dried cuttle fish (Squid), with $\leq$ 19% moisture content, when treated with an irradiation dose of 2-3 kGy and stored at 20-24 °C, maintained good quality for up to 9 months (*18*).

- Crustacea
  - Precooked packaged crabmeat (Blue crab, Dungeness, King crab) receiving 0.5-2.5 kGy gamma irradiation was reported to have an extended shelf life of 2-3 weeks beyond the normal 7 days (*19, 20, 21, 22, 23, 24*).
  - Optimum doses for both American and European lobsters were reported to be 0.75 kGy and 1-3 kGy, respectively (*25, 26, 27*).
  - Numerous studies conducted on a variety of shrimp genera have shown that low dose irradiation of 1.5-2.5 kGy will extend shelf life for up to 2 weeks beyond normal with no change in sensory quality (*7, 23, 28, 29, 30, 31, 32*).

- Marine Finfish
  - Numerous species of finfish (tuna, whiting, sole, ocean perch, etc.) were studied from the late 1950's through the 1980's. In general, marine species, receiving irradiation doses of 1.5-2.5 kGy and stored under refrigeration (0-1°C), exhibited a 25-30 day shelf life (*33, 34, 35, 36*).
  - More recently, Thibault and Charbonneau (*37*) determined that both fresh and frozen Atlantic cod fillets could be stored under refrigeration for 28 days with the optimal irradiation dose of 2-3 kGy.
  - Several species of fish with higher fat content (mackerel, salmon), when processed with irradiation doses at >1.5 kGy, demonstrated an extended shelf life in relationship to spoilage but did not have good sensory qualities due to lipid oxidation. (*38, 39*).
  - Levels of biogenic amines typically increase during fish spoilage and are a major indicator of soured fish. Mendes et al. (*40*) reported that levels of biogenic amines in Blue Jack Mackerel were significantly reduced by irradiation processing at 1, 2, and 3 kGy doses compared to control samples.

- Freshwater Finfish
  - Catfish have an optimum irradiation dose of about 2 kGy. At higher levels, the fatty portion of the fish will undergo lipid oxidation and effect the overall quality of flavor. Storage stability for good quality catfish was about 12 days beyond the normal 5 days (*41*).
  - Other fattier species such as chub, mullet, lake herring, and trout,

when irradiated with low does (1kGy or less), obtained a week of shelf-life extension (*42, 43, 44, 45, 46*).

- Early work on irradiation processing of salmon fillets determined this species to be an unlikely candidate for irradiation processing due to high fat content and dark astaxanthin pigmentation. During irradiation processing, even at low doses of 1-2 kGy salmon undergo rapid lipid oxidation and lose their pigmentation through bleaching (*27, 47*).

Pathogenic Microorganisms

Research with gamma irradiation has focused primarily on low-dose pasteurization of fish and shellfish. This historical approach was fostered by the U.S. Atomic Energy Commission, which chose to work primarily with pasteurization dose levels on fresh seafood products (*4*). The principal reason that pasteurization levels were chosen was that dosages close to or above 10 kGy will definitely affect the original sensory and physical qualities of seafood, eliminating their being sold as "fresh" product. Advantages of low-dose pasteurization included control or elimination of many pathogens and parasites as well as increasing the shelf-life by an average of 7-10 days.

Pathogenic bacteria find their way into seafood products in different ways. The pathogens of primary concern are those found naturally in the fresh water or marine environment and include various *Vibrio* spp., *Aeromonas* and *C. botulinum* type E. *Vibrio* species have proven relatively sensitive to low dose gamma irradiation processing compared with other pathogens. Table II demonstrates the sensitivity of *Vibrio cholera* in shrimp, crabmeat, and crawfish. Pathogenic strains of *Vibrio vulnificus* and *Vibrio parahaemolyticus* have shown similar responses in shell stock oysters, Figures 1, 2, 3 (*13, 14, 15*). However, oysters did not have extended shelf-life following irradiation processing in any of these studies. Use of irradiation as a post harvest process to eliminate the risk or *Vibrio vulnificus* is the major justification for use of this process. *Vibrio parahaemolyticus* 03:K6 (10^5/cfu/g oyster meat) is the most process resistant of all the *Vibrio* pathogens tested to date, but is reduced to nondetectable levels following 1.5 kGy gamma irradiation (*15, 48*). Environmental strains of *Vibrio,* in shrimp or crawfish are more sensitive than the pathogenic strains and are reduced to nondetectable levels with <1 kGy gamma irradiation (*15*). In fresh Gulf shrimp inoculated with 10^7cfu/g of *Vibrio parahaemolyticus* 05:17, an irradiation dose of at least 0.3 kGy was needed to reduce this bacterium to nondetectable levels. At lower dosages of 0.05, 0.1, 0.15, and 0.25 kGy, respectively, as many as 10^3 cfu/g of *Vibrio* survived during ice storage for up to 3 weeks (*49*).

A study by Palumbo et al. (*52*) examined the radiation resistance of *Aeromonas hydrophilia*, a psychrotrophic pathogen of emerging importance. The results of this study indicated that a pasteurizing dose of ionizing radiation

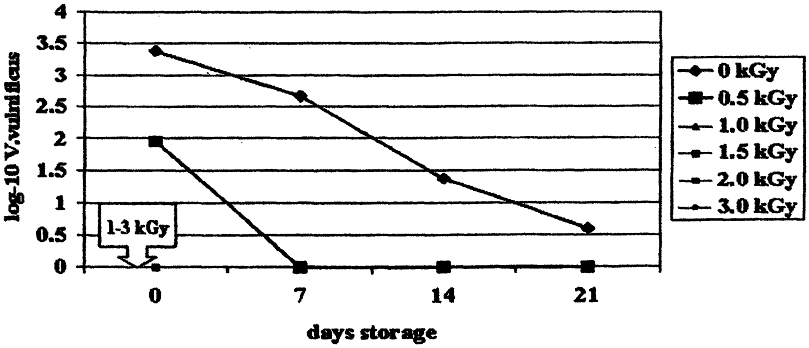

Figure 1: Irradiation response of naturally occurring *Vibrio vulnificus* in shell stock oysters.

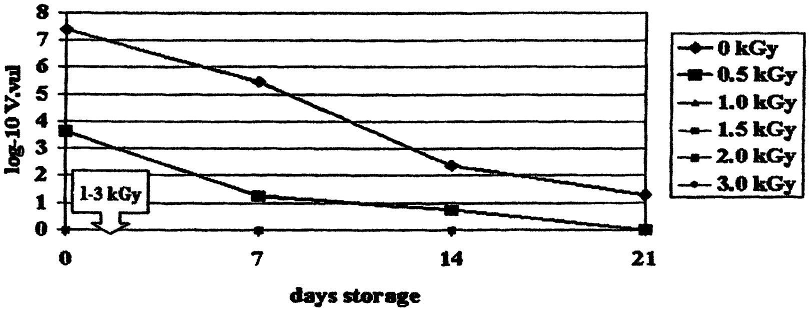

Figure 2: Irradiation response of *Vibrio vulnificus* inoculated in shell stock oysters

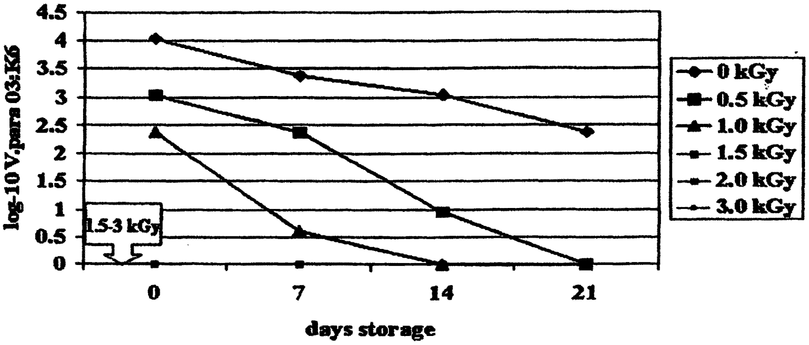

Figure 3: Irradiation response of artificially inoculated *V. parahaemolyticus 03:K6* in shell stock oysters

Table II. Response of *Vibrio cholera* to low dose gamma irradiation (*4*)

Product	*Dose (kGy)*	*Log $_{10}$ cfu/g*[c]
Shrimp[a]	*0 (control)*	*7.0*
	0.5	2.0
	1.0	Negative
Crabmeat[b]	0 (control)	7.0
	0.25	3.0
	0.50	Negative
	1.00	Negative
Crayfish[b] (crawfish)	0 (control)	7.0
	0.25	5.5
	0.50	3.5
	1.00	Negative

[a] Reference No. 50
[b] Reference No. 51
[c] cfu/g = colony forming units/gram seafood

at 1.5 kGy was sufficient to destroy *A. hydrophilea* in concentrations of $\leq 10^5$ cfu/g when present in retail fresh fish such as bluefish (*4*). *Clostridium botulinum* type E has always been a potential problem in seafood products since it is found naturally in the coastal environments and produces *Botulinum* toxin under certain storage conditions or product abuse. Spores of this bacterium inoculated at 10^3 and 10^4 spores/g into fresh Gulf shrimp and irradiated at a dose of 1.5 kGy produced no toxin during 31 days of iced (0^0C) storage (*4*). However, when the same inoculation treatment and irradiation at 1.5, 2.0, 3.0 and 5.0 kGy doses were given to shrimp packaged under vacuum and stored at 6^0C, botulinum toxin was produced in all samples after 7 days except those treated with 5.0 kGy dose. At the higher level, toxin was not produced until 30 days of storage (*53*).

Seafoods may become exposed to human intestinal bacterial pathogens if sewage wastewater is present in their growing environments. The greatest concern is with species of *Salmonella, E. coli, Enterococcus, Shigella,* and *Listeria monocytogenes*. *Listeria monocytogenes,* in pure broth culture, at concentrations of 10^3cfu/ml, were destroyed with <2.0 kGy gamma irradiation dose (*54*). In crayfish (crawfish) tailmeat, 10^4 cfu/g concentrations of *Listeria monocytogenes* were eliminated with <3 kGy gamma irradiation with no adverse effect on the sensory quality of the crawfish. Higher doses of >3 kGy did affect the texture of the meat by taking on a firmer protein texture, much like heat cooking (*55*). Juneau (*56)* reported that *Listeria monocytogenes* in crabmeat at 10^7 cfu/g were still viable after receiving 2 kGy gamma irradiation processing. Of a future concern is the introduction of *Listeria monocytogenes*during processing to products such as smoked fish. Research studies on the radiation sensitivity of *L. monocytogenes* in smoked fish are currently being conducted (*57*). *Salmonella* spp response to irradiation processing was part of the early research on seafood irradiation. *Salmonella typhimurium*, when inoculated into Louisiana Gulf oysters, was not recovered after 14 days of ice storage when treated with 2 kGy Cobalt-60, gamma irradiation (*58*). Underal and Rossebo (*59*) recommended a dosage of 13 kGy when attempting to reduce *Salmonella senftenberg* in Norwegian fish meal by 10^8 cfu/g; even though *Salmonella* in commercial fish meal seldom exceeds 10^1 cfu/g. *Escherichia coli* is most often used as an indicator of fecal pollution in marine and freshwater environments. Lee (*60*) reported that *E. coli*, when inoculated at 10^4 cfu/g in shrimp and oysters, had a 5% survival rate at 1 kGy and 0.1% at 2 kGy dose. It appears that >2 kGy dose of irradiation would be required to eliminate *E. coli* at these concentrations. *Enterococci* have shown a similar irradiation response requiring >2 kGy for elimination from shrimp and oysters (*61*). With an initial concentration of 10^6 cfu/g, *Streptococcus faecalis* was reduced by 4 and 5 log with 1 kGy and 2 kGy, respectively.

Another concern is the introduction, by processing personnel, of *Staphylococcus aureus* into seafood during packaging and handling. Following 1 kGy irradiation, *S. aureus* was reduced by 4 logs in fresh Gulf shrimp stored for 21 days (*62*). Another study reported that *Staphylococcus* in dried and smoked mackerel required as much as 5 kGy to be inactivated (*63*). Likely due to the low water activity of these products, there were no adverse sensory changes noted.

Viruses in general are among the most radiation resistant pathogenic microorganisms. In most instances, cooking seafood exposed to viruses eliminates the risk of viral infection. However, with raw shellfish the potential for viral contamination remains a concern. In the winter of 1996-1997, the Norwalk virus emerged as a major viral contaminant in shellfish growing areas in South Louisiana. In general, results of viral remediation in shellfish by irradiation have not proven to be successful. Levels of irradiation, sufficient to kill the viral particles, render the product cooked or inedible. In a typical study, Girolamo et al. (*64*) reported that *Poliovirus* inoculated into West Coast oysters were relatively unaffected by up to 5 kGy irradiation processing. Inactivation of pathogenic viruses in fish and shellfish requires doses that are too high to be usable or even generate interest for this usage by the food industry (*5, 65*).

Fish parasites, such as *Anisakis*, are normally controlled by freezing the fish prior to processing. Irradiation processing at high doses (>5 kGy) is required to eliminate parasites, and has not been considered a viable process option for fish used for raw consumption. Van Mameren et al. (*66*) reported irradiation doses of 6 kGy in herring were needed to inactivate *Anisakis* in the fish flesh. At this dosage, the fish were unacceptable in appearance and flavor. Other parasites in fish are usually destroyed by cooking. Parasites responsible for poor quality fish flesh, such as the protozoan Kudoa, have not been studied.

Consumer Acceptability of Irradiated Seafood

Low dose gamma irradiation has proven to be effective in reducing pathogenic and spoilage microorganisms in a variety of seafood products. However, little analytical information is available on the effects of irradiation processing on the sensory quality and consumer acceptance of such products. Chen et al. (*19*) compared microbial and sensory quality of irradiated (2 kGy or less) prepackaged crab products (white lump, claw, and fingers) through a 14-day iced storage. Irradiation effectively reduced spoilage bacteria extending shelf-life by more than 3 days beyond the control samples. During storage, fresh crab odor and flavor were similar for treated and control samples, while off-flavors from spoilage developed more rapidly in control samples. Overall acceptability scores for irradiated crab samples were higher than for control samples throughout the 14-days iced storage. Andrews et al. (*15*) reported that in-shell oysters treated with 1 kGy irradiation were highly acceptable to consumers, and in fact, consumers were not able to distinguish the irradiated oyster from the raw oyster control samples. Following a consumer survey conducted at the Boston Seafood Show in 2002, Posadas et al. (*67*) reported that nearly 20% of oyster nonconsumers would be willing to purchase irradiated oysters due to the enhanced safety. Of those surveyed, 90-95% did not believe irradiation of oysters would make the oysters radioactive or have any adverse affect on the sensory and nutritional quality of oysters.

Conclusion

In spite of the extended research reported on seafood irradiation processing during the last 45 years and that irradiated seafood is available in Asian and European markets, approval by the United States Food and Drug Administration is still pending. Currently, there are two active petitions pending in the FDA Office of Food Additive Safety (previously the Office of Premarket Approval). The first is for molluscan shellfish and the second is for crustaceans. A third petition for fish is being finalized for submission in 2003 (*68*). There is currently a great deal of industry interest among United States processors for obtaining approval for the use of irradiation processing in shellfish and fish.

Irradiation processing, when used properly, can protect the consumer from many microorganisms of public health concern. These processes have been shown to maintain fresh sensory qualities and nutritive value for a variety of seafood products. In addition, fresh seafood products treated with irradiation have a longer shelf-life and therefore can be marketed to areas far removed from the harvesting areas.

Authors Note: This report is an attempt to represent the wide scope of research that has been conducted in the realm of seafood irradiation processing. It is submitted as a summary of known information. There are currently several universities and US government agencies continuing to research the effective use of irradiation processing on fish and shellfish. It is believed that the approval for irradiation processing of molluscan shellfish will be granted in 2003 (*69*).

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Chapter 11

Application of Electron Beam to Surimi Seafood

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Color, texture, microbial inactivation, and protein-protein interactions of surimi seafood subjected to electron beam (e-beam) were investigated. Color whitening and stronger gel of surimi seafood was measured. The D_{10} value for *Staphylococcus aureus* was 0.34 kGy. Modeling of microbial inactivation demonstrated that two-sided e-beam may control *S. aureus* if the surimi seafood package is thinner than 82 mm. SDS-PAGE showed gradual degradation of myosin heavy chain (MHC) as e-beam dose increased. Degradation rate was slower when frozen samples were treated. The integrity of actin (AC) was slightly affected by e-beam.

The Centers for Disease Control and Prevention (CDC) estimated that food-borne diseases in the USA annually cause 81 million illnesses, 616,377 hospitalizations, and 5000 deaths. Food-borne disease outbreaks implicating seafood are 5, 19, 4, and 12 times as frequent as outbreaks linked to beef, pork, chicken, and turkey, respectively (*1*).

The Food and Drug Administration (FDA) established a "zero tolerance" for *Listeria monocyotogenes*, *Salmonella*, *Vibrio cholerae*, *Vibrio vulnificus*, and for the presence of toxin, viable spores, or vegetative cells of *Clostridium botulinum* in ready-to-eat fishery products. If enterotoxigenic *Escherichia coli* are present at 1 x 10^3/g or *Vibrio parahaemolyticus* at 1 x 10^4/g, the FDA will consider regulatory action. The product may be recalled if it tests positive for staphylococcal toxins or if 1 x 10^4/g of *Staphylococcus aureus* are present.

Fishery products, like other muscle foods, require good pasteurization practices to maintain microbial safety (*2*, *3*). Psychrotrophic microflora, inherent to seafood, grows well under refrigeration conditions. Therefore, fishery products are particularly susceptible to microbial deterioration.

The surimi seafood industry traditionally uses hot water or steam as a pasteurizing medium (*4*). However, various pasteurization regimes are used (*5*, *6*). Consequently, products may be overcooked, resulting in undesirable changes of quality (*7*). It is feasible though to determine a point at which minimum loss of physicochemical quality and desired microbial inactivation occur simultaneously (*8*). However, some quality deterioration is still inevitable (*8*, *9*). Current thermal pasteurization methods, therefore, may be inadequate to simultaneously maintain microbial safety and product quality. Consequently, in the wake of the September 11 incident, it is increasingly important that the food industry take appropriate action to assure a continuous risk-free food supply (*10*, *11*).

Electron beam (e-beam), in contrast to thermal pasteurization, utilizes high-energy electrons for pasteurization or sterilization. Electrons are accelerated to the speed of light by a linear accelerator. Then, electrons are passed through the food product, inactivating bacteria. The electron source is electricity and, unlike gamma radiation, e-beam does not use radioisotopes (*12*). E-beam enables the application of high dose rates (e-beam, 10^3-10^5 Gy/sec; gamma, 0.01-1 Gy/sec), resulting in a short exposure time (*13*). E-beam processing does not affect the temperature of processed food. Therefore, e-beam is likely to minimize the degradation of food quality (*14*).

E-beam, unlike gamma rays, has limited penetration depth (*15*), which may affect microbial inactivation depending on the package size. The overall antimicrobial effects, though, of gamma rays and e-beam are comparable (*15*, *16*).

Joint Expert Committee on Food Irradiation representing FAO/IAEA/WHO (Food and Agriculture Organization/International Atomic Energy Agency/World

Health Organization) concluded that irradiation of any food up to 10 kGy caused no toxicological hazards and introduced no nutritional or microbiological problems (*17*). Application of e-beam to surimi seafood has not been reported.

Our objectives were to determine color and texture, microbial inactivation, and protein-protein interactions of surimi seafood subjected to e-beam.

Materials and Methods

Color

Un-pasteurized surimi seafood crabsticks were obtained from Louis Kemp/Bumble Bee Seafoods (Motley, MN). Sticks were tightly placed on plastic trays and sealed in plastic pouches made of a 3-mil (76 microns) standard barrier nylon/PE film (Koch, Kansas City, MO) under either aerobic (non-vacuum pack) or anaerobic (vacuum pack) conditions.

Before e-beam treatment (Ion Beam Applications, San Diego, CA), the temperature of the sticks was equilibrated to either −18, 5, or 23°C. The sticks were exposed to four doses of e-beam (0, 1, 2, and 4 kGy) with energy fixed at 10 MeV.

Tristimulus color values L* a* b* were measured using a Minolta chroma meter CR-300 (Minolta Camera Co. Ltd., Osaka, Japan) (*18*). The external (red-colored) layer of the crabstick was peeled off and the remaining stick was ground to a paste. The paste was transferred onto a Petri dish and packed compactly inside for color measurement. To eliminate the effect of compactness on color values, the same amount of paste was applied for each measurement. Color measurement of at least three sticks was taken with at least five measurements per crabstick.

A paired t-test based on the pooled standard deviation was used to determine differences between means of various treatments (*19*).

Texture

Frozen Alaska pollock surimi was tempered and cut into small chunks. Surimi chunks were chopped in a silent cutter (Model UMC5, Stephan Machinery Corp., Columbus, OH) at low speed for 1 min. Salt (2 %) was added and the surimi paste was chopped at low speed for 0.5 min. Final moisture content was adjusted to 78% by adding ice to the paste, followed by chopping at low speed for 1 min. High speed chopping under vacuum (0.5 bar) was applied for the last 3 min. During chopping, the temperature was kept below 5°C. The paste was then stuffed into torsion gel molds and cooked at 90°C for 15 min, which resulted in hourglass-shaped surimi gels (length = 2.9 cm, end diameter = 1.9 cm, and minimum diameter = 1.0 cm).

Surimi gels were subjected to one-sided e-beam (0, 1, 2, 4, 6, 8, 10, and 25 kGy) with energy fixed at 10 MeV (Ion Beam Applications, San Diego, CA).

Surimi gels were kept at room temperature for 2 h prior to measurement. Hourglass-shaped gels were glued to plastic discs and subjected to torsional shear using a Hamman Gelometer (Gel Consultant, Raleigh, NC) set at 2.5 rpm. Shear stress and shear strain were measured at mechanical fracture to determine gel strength and gel cohesiveness, respectively (*20*). At least five measurements per e-beam dose were taken.

Microbial Inactivation

Electron Penetration

Surimi gels were prepared as described in "Texture" except that the paste after chopping was not stuffed into torsion gel molds but the paste was placed in a waxed cardboard box (4 cm x 4 cm x 20 cm) and the air gaps were carefully removed. The boxes were vacuum packed and cooked in a water bath at 90°C for 45 min. Immediately following cooking, the surimi gels were cooled in ice slush. Surimi gels with two different dimensions were prepared: (1) 3 cm x 3 cm x 7 cm, and (2) 3 cm x 3 cm x 9 cm.

Surimi gels were subjected to two doses (3 and 20 kGy) using one-sided e-beam with energy fixed at 10 MeV (Ion Beam Applications, San Diego, CA). The experiments were performed in duplicate.

Dosimeters (calibrated radiochromatic dye films) were distributed every 1 cm from the top surface to the bottom of the surimi gels. Exposed dosimeters were read by a spectrophotometer at 605 nm and the doses absorbed at their respective locations were calculated. Absorbed doses were plotted against distance between dosimeters and the surimi gel surface, creating a dose map. The dose map allowed determination of R_{opt} (depth of surimi gel at which the absorbed dose equaled the dose at the surface of the surimi gel), R_{50e} (depth of surimi gel at which the absorbed dose has decreased 50 % of the absorbed dose at the surface of surimi gel), R_{max} (depth of surimi gel at which the absorbed dose reached its maximum value), and R_{50max} (depth of surimi gel at which the absorbed dose decreased 50 % of its maximum value). A polynomial regression equation was fitted to the experimental data. Microsoft Excel was used for the calculations. A paired t-test based on the pooled standard deviation was used to determine differences between means of various treatments (*19*).

Microbial Inactivation

Surimi seafood crabsticks (hereinafter surimi seafood) were obtained from a commercial factory (Louis Kemp/Bumble Bee Seafoods, Motley, MN). The sticks were ground into a paste. The paste was placed on plastic trays and inoculated (5%) with a cocktail of six strains of *Staphylococcus aureus*, followed by incubation at 37°C for 72 h, resulting in a final concentration of 10^9 CFU/g. Following incubation, the inoculated paste was packed in a plastic pouch made of 3 mil (76 microns) standard barrier nylon/PE film (Koch, Kansas City, MO). A half of the packages was anaerobically packed (vacuum); the other half was aerobically packed (non-vacuum). The samples (23°C) were subjected to four doses (0, 1, 2, and 4 kGy) of one-sided e-beam with energy fixed at 10 MeV (Ion Beam Applications, San Diego, CA). Following the e-beam treatment, the survivors were enumerated.

The six strains of *S. aureus* were from Dr. M. A. Daeschel's collection (Oregon State University, Corvallis, OR) and identified as 138-cps, 146-cps, 153-cps, 648-gf, 649-gf, and 657-gf. The strains were stored at -70°C. Before inoculation in the paste, the strains were grown in staphylococcus broth (Difco Laboratories, Detroit, MI) at 37°C for 24 hr in an incubator shaker set at 200 rpm. In our preliminary experiments, it was determined that under these conditions each strain reached a stationary phase of growth and concentration of 10^9 CFU/g (data not shown).

Enumeration of *S. aureus* survivors was performed on staphylococcus 110 agar (Difco Laboratories, Detroit, MI) by a serial 10-fold dilution using the spread plating method (*21*). Following e-beam treatment, before the survivors were enumerated, the samples were thoroughly mixed in order to obtain uniform distribution of survivors. Bacterial enumeration was performed in triplicate. The presence of *S. aureus* was confirmed by gram staining, catalase, and coagulase tests. The tests were performed according to the manufacturer (Difco Laboratories, Detroit, MI). A paired t-test based on the pooled standard deviation was used to determine the differences between means of various treatments (*19*).

Predictive Model of Microbial Inactivation by E-Beam

Survivors were plotted on a logarithmic scale as a function of dose, resulting in a survivor curve (*22*). D_{10} value defined as the dose in kGy necessary to reduce the microbial population by 90 % (1 log) (*22*), was calculated as a negative reciprocal of the slope of the survivor curve (*23*, *22*) (eq 1).

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{D} * t \tag{1}$$

N – number of survivors at e-beam dose,
N_0 – initial microbial concentration,
D – D_{10} value, decimal reduction dose,
t – e-beam dose.

Dose absorbed, as a function of surimi seafood thickness, was simulated by the polynomial equation obtained from the dose map. The dose absorbed was related to the D_{10} value, resulting in a total log reduction of *S. aureus* in the surimi seafood. The number of *S. aureus* (CFU/g) that survived e-beam treatment was calculated based on the total log reduction and the initial number of *S. aureus* (CFU/g). Microsoft Excel was used for the calculations.

Protein-Protein Interactions

Alaska pollock surimi (hereinafter called surimi) and surimi gels were used in this experiment. Surimi gels were prepared as described in "Texture".

Half of the surimi gels and surimi was at room temperature (23°C) when subjected to e-beam. The other half was frozen (−18°C) when subjected to e-beam. Surimi gels and surimi were subjected to 0, 1, 2, 4, 6, 8, 10, and 25 kGy of one-sided e-beam with energy fixed at 10 MeV (Ion Beam Applications, San Diego, CA).

Surimi (3 g) or surimi gel (3 g) was solubilized in 27 mL of 5 % sodium dodecyl sulfate (SDS) solution (*24*). Collected supernatant was analyzed for protein concentration by the Lowry assay (*25*). Samples were diluted by 50-fold so that residual SDS did not interfere with the Lowry assay.

Protein concentration was adjusted to 2 mg/mL, and then mixed with 5x sample buffer (1 M Tris-HCl (pH 6.8), 50 % glycerol, 10 % SDS, 14.4 mM β-mercaptoethanol (β-ME), 1 % bromophenol blue, and distilled deionized (dd) H_2O), followed by heating at 90°C for 5 min (*26*). Aliquots of 12.5 µL (25 µg) of proteins per well were used for SDS-PAGE. Discontinuous (12 %

October 23, 2009 | http://pubs.acs.org
Publication Date: January 2, 2004 | doi: 10.1021/bk-2004-0875.ch011

polyacrylamide separating and 5 % polyacrylamide stacking gel) SDS-PAGE under denaturing conditions at 200 mA of constant current were performed (*26*). The electrophoretic patterns of proteins were stained with Coomassie brilliant blue R-250 (Bio-Rad, Richmond, CA), followed by destaining with solution containing 25 % ethanol and 10 % acetic acid.

Results and Discussion

Color and Texture

The L* and a* values were not affected (P>0.05) by e-beam. The b* value of the crabsticks decreased (P<0.05), resulting in whiter color (Figure 1). In general, whiter surimi seafood crabsticks denote better color quality. Color browning has been reported as a result of subjecting the crabsticks to heat (*7*). Ozone, which may be generated during e-beam processing (*27*), might have bleached the yellow hue, resulting in a reduced b* value. Sample temperature during e-beam treatment and oxygen availability did not affect color (P>0.05).

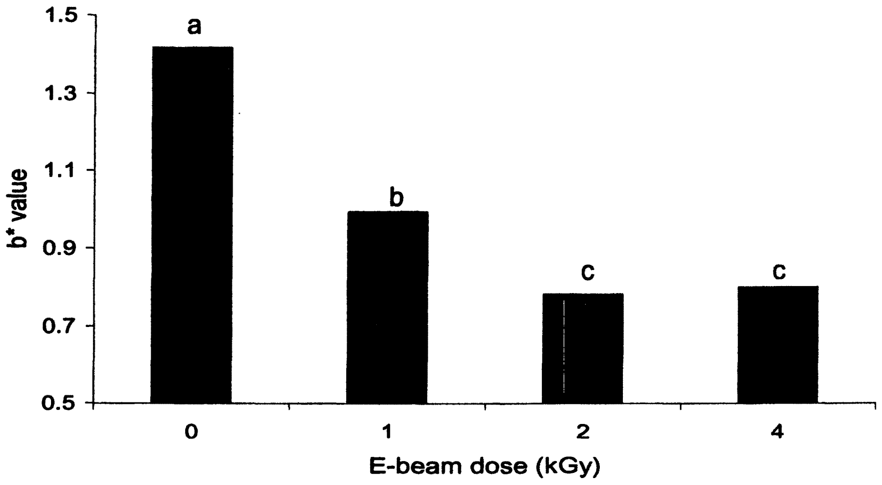

Figure 1. The b value (yellowness) as affected by e-beam (different letters on the bars indicate a significant difference at P<0.05).*

Shear stress of surimi gels increased proportionally to e-beam dose up to 6-8 kGy, and then decreased (Figure 2). Shear strain of surimi gels was not affected by e-beam. Shear stress and shear strain indicate the strength and cohesive nature of surimi gels, respectively (*28*). Therefore, the results suggest that e-beam treatment up to 6-8 kGy improved the strength of surimi gels.

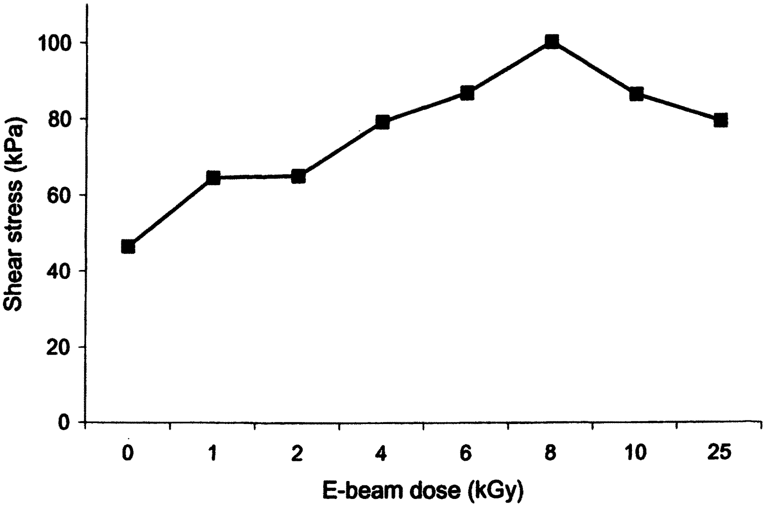

Figure 2. Effect of e-beam on shear stress of surimi gels.

Similar trends have been reported in literature. Gamma radiation applied to precooked lobster resulted in increased gel strength (*29*). Increased shear resistance of chicken muscle treated with gamma radiation was measured using Kramer shear cell (*30*).

Comparable textural changes were also reported for other muscle foods irradiated with ultraviolet (UV). Increased strength of sardine, beef, and pork surimi gels subjected to UV was measured (*31*). Similar results were reported for gels developed from mackerel actomyosin, and from sardine and pork pastes (*32, 33*).

Microbial Inactivation

Figure 3 (*34*) shows the dose map for surimi gels. The absorbed dose increased up to 2 cm deep from the gel surface. Then absorption gradually decreased, reaching a minimum value at approximately 5 cm from the gel surface. The surimi gels used in our experiments had 78% moisture. At 78% of moisture and a temperature of 30°C, surimi gels had a specific density of 1.067 g/cm^3 (*35*).

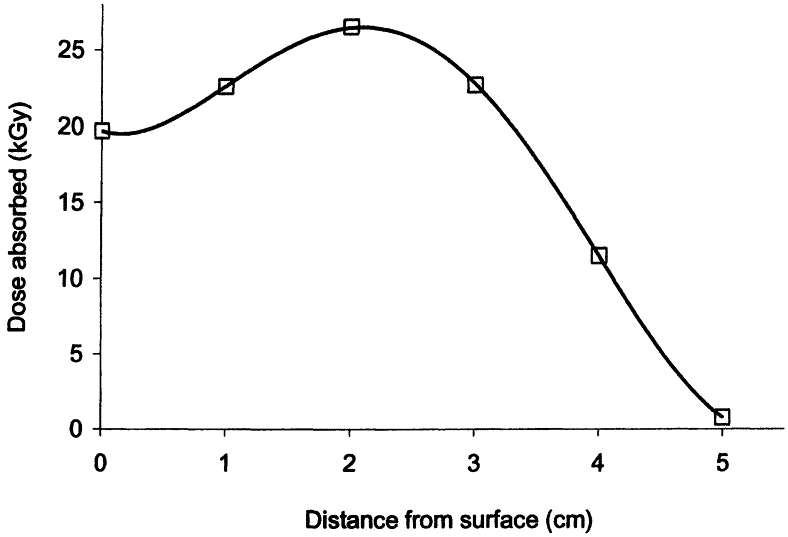

Figure 3. Dose map for one-sided e-beam for surimi gels (Reproduced with permission from reference 34. Copyright 2003 J. Food Sci.)

Similar results can be found in literature. E-beam at 10 kGy and energy fixed at 10 MeV applied to aqueous solutions resulted in 12.5 kGy being absorbed 2 cm from the surface and no absorption below 5 cm (*27*). The increased absorption under the surface of irradiated product has been attributed to the formation of secondary electrons that, because of their lower energy, are more effectively absorbed than the primary electrons (*27*).

The dose absorbed (kGy) followed a polynomial function (Dose Absorbed = $1.76x^4-17.73x^3+43.14x^2-12.32x+99.96$, x – distance from the product surface) regardless of the dose applied and product thickness. In our experiments, e-beam at 3 and 20 kGy was applied to 7 and 9 cm thick surimi gels. In all cases, the dose absorbed followed the same polynomial function ($P>0.05$).

Based on the dose map, the R_{opt}, R_{max}, R_{50e}, and R_{50max} were calculated as 33, 21, 41, and 39 mm, respectively. According to the R_{50e}, two-sided e-beam can efficiently penetrate surimi seafood up to 82 mm thick, resulting in the dose absorbed across the entire thickness as being equal to or higher than the dose applied. This finding is in accordance with literature, which suggests that e-beam could be applied to food up to 8-10 cm thick that has a specific density of 1 g/cm^3 (*27*).

E-beam at 1, 2, and 4 kGy resulted in 2.9-log reduction, 6.1-log reduction, and no detectable colonies of *S. aureus* in surimi seafood, respectively (Table I). The D_{10} value was 0.34 kGy. Effects of radiation are linear with dose, up to 15 kGy (*36*). Therefore, application of 4 kGy in our tests may have resulted in a 12-log reduction, as verified by no colonies at 4 kGy (Table I). Our D_{10} value for *S. aureus* in surimi seafood was similar to the D_{10} value of 0.29 kGy reported for shrimp (*27*). Oxygen unavailability under vacuum packing did not affect microbial inactivation ($P>0.05$).

Table I. Effect of E-Beam on Inactivation of *S. aureus* in Surimi Seafood

E-beam dose (kGy)	*S. aureus count (CFU/g)*	*Log reduction*
0 (control)	1.2×10^9	0
1	2.7×10^6	2.9
2	2.7×10^3	6.1
4	Not Detectable	≈12

Figure 4 shows simulations of dose absorbed (Figure 4, left) and inactivation of *S. aureus* (Figure 4, right) in surimi seafood subjected to e-beam. The dose absorbed in surimi seafood was simulated using the polynomial function obtained from the dose map. By applying the D_{10} value for *S. aureus* in surimi seafood to the simulated dose absorbed, total log reduction was estimated. Final concentration of *S. aureus* (CFU/g) was obtained by applying the total log reduction to the initial concentration of *S. aureus* (CFU/g). The simulations in Figure 4 are based on 90 mm sample thickness, 2 kGy applied dose, 0.34 kGy D_{10} value for *S. aureus* in surimi seafood, and 2.3×10^5 CFU/g initial concentration of *S. aureus*.

Based on R_{opt} and R_{50e} equaling 33 and 41 mm, respectively, one-sided and two-sided e-beam can efficiently penetrate 33 and 82 mm of surimi seafood, respectively. Efficient penetration is defined as a penetration that results in the dose absorbed in entire surimi seafood equal to or greater level than the dose applied. Therefore, two-sided e-beam represents better utilization of the dose applied.

The dose absorbed below 33 mm for one-sided e-beam would be lower than the dose applied, thereby, the desired antimicrobial effect would not be obtained.

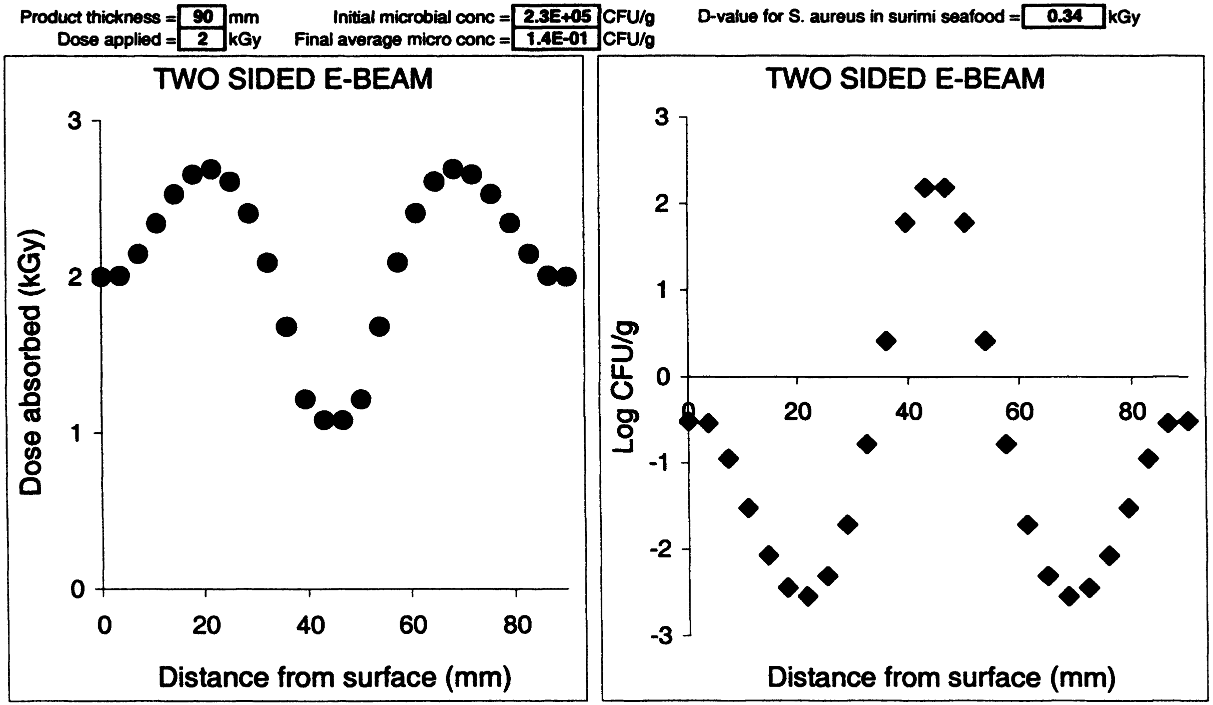

Figure 4. Predictive models for dose absorbed (left) and inactivation of S. aureus (right) in surimi seafood by two-sided e-beam. This figure represents model predictions under given conditions: product thickness 90 mm, dose applied 2 kGy, D_{10} value for S. aureus in surimi seafood 0.34 kGy, initial population of S. aureus 2.3 x 10^5 CFU/g.

Simulation of dose absorbed and microbial inactivation by two-sided e-beam for surimi seafood thicker than 82 mm demonstrates under-processing starting at a depth of 33 mm from the top and bottom surfaces (Figure 4). If the thickness of surimi seafood processed with two-sided e-beam exceeds 82 mm, then the maximum under-processing occurs at the geometrical center of the package.

This geometrical center may be referred to as the "cold spot". The term "cold spot" is commonly used to describe under-processed areas of food processed with non-uniform heating (i.e., microwave or radio frequency). However, it is obvious that e-beam processing does not generate heat. The analogy refers only to under-processing, which may result in elevated microbial survival in the under-processed areas of the product. Consequently, one-sided and two-sided e-beam processing are not recommended for surimi seafood thicker than 33 and 82 mm, respectively.

Protein Degradation

The SDS-PAGE in 12 % polyacrylamide gel (Figure 5) showed gradual degradation of myosin heavy chain (MHC). The degradation was proportional to the increase of e-beam dose. Gradual disappearance of MHC resulted in a subsequent increase of smaller molecular weight proteins (200 to 50 kDa) that appeared in each lane below MHC. The complete disappearance of the MHC band was observed at 25 kGy for raw surimi and surimi gels subjected to e-beam while at 23°C. However, raw surimi and surimi gels subjected to 25 kGy while at −18°C showed a very thin MHC band, suggesting slower degradation at the lower temperature. Actin (AC) and other fractions of myofibrillar proteins were not affected by doses from 0 – 10 kGy and marginally affected at 25 kGy.

Similar observations have been reported in literature (*32*, *37*, *38*). A gradual disappearance of a band associated with the main chain (210 kDa) of myosin subjected to gamma radiation was observed using SDS-PAGE (*37*). A slower rate of myosin degradation when frozen samples were subjected to radiation was also observed (*37*). A cross-linking of mackerel actomyosin induced by UV radiation was observed using SDS-PAGE (*32*). The UV radiation caused gradual disappearance of MHC in flying fish (*38*).

In our experiments, electrophoresis was conducted under denaturing conditions of β-ME and SDS. If e-beam had induced cross-linking by disulfide bonds or hydrophobic interactions, they would have not been seen due to cleavage of those bonds by β-ME and SDS, respectively. However, cross-linking that involves bonds other than disulfide bonds or hydrophobic interactions would have been detected. Therefore, it is suggested that e-beam did not induce cross-linking other than disulfide bonds or hydrophobic interactions.

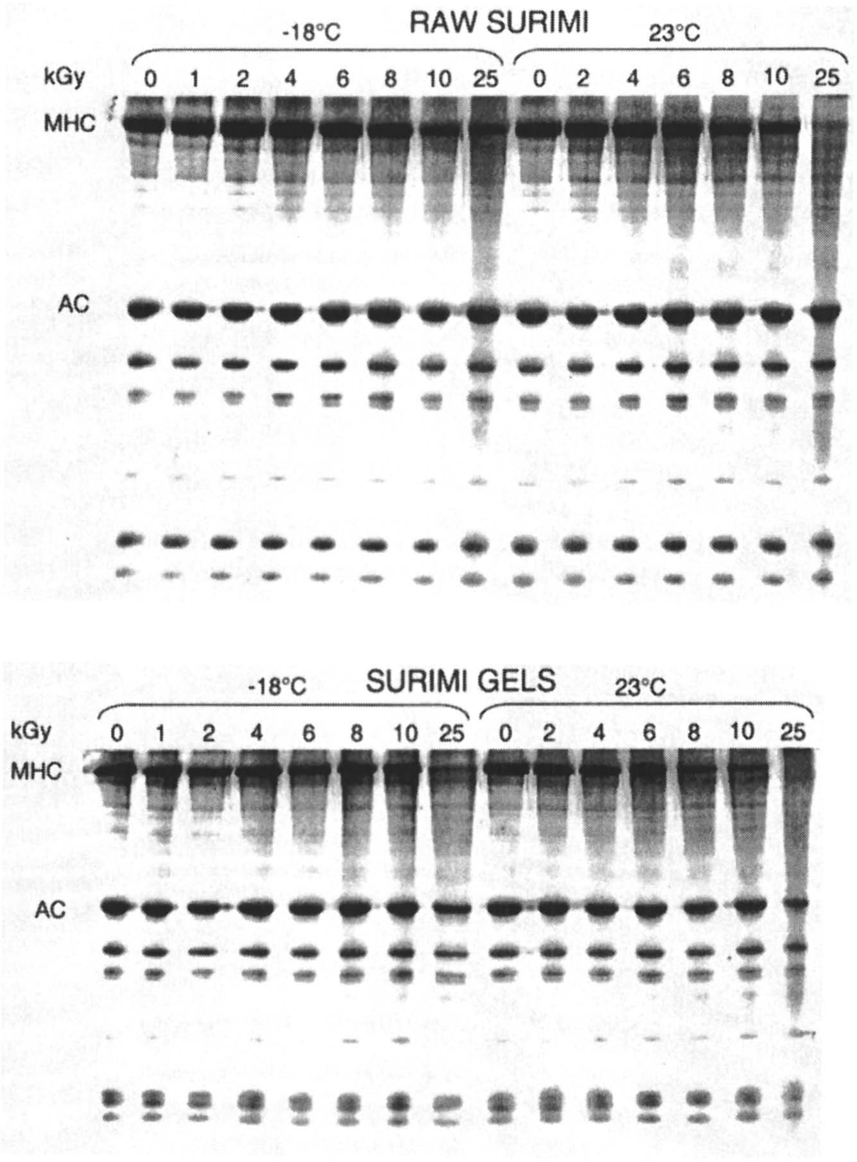
RAW SURIMI
-18°C
23°C
kGy
0 1 2 4 6 8 10 25
0 2 4 6 8 10 25
MHC
AC
SURIMI GELS
-18°C
23°C
kGy
0 1 2 4 6 8 10 25
0 2 4 6 8 10 25
MHC
AC

Figure 5. SDS-PAGE of Alaska polock surimi (left) and surimi gels (right) applied to 12 % polyacrylamide gel at 25 μg of proteins/well. Sample temperature was -18°C (top) and 23°C (bottom) during e-beam treatment. MHC – myosin heavy chain, AC – actin.

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Chapter 12

Irradiation of Prepackaged Food: Evolution of the U.S. Food and Drug Administration's Regulation of the Packaging Materials

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The FDA approved the first materials intended for use as packaging for irradiated foods (polyolefin films, polystyrene, cellophane, vinylidene chloride copolymers, and others) in 1964. Several other materials were approved for this use during the next four years. Since then, only one material, ethylene vinyl acetate copolymer, was added to Title 21 of the *Code of Federal Regulations*, in 1989. The recent interest in irradiating meat to eliminate pathogens such as *E. coli* O157:H7 has resulted in several industry submissions to the Agency regarding new packaging materials, as well as the radiation sources, intended for use during the irradiation of prepackaged food. A brief history of FDA regulation of packaging materials irradiated in contact with food, including a discussion of human exposures to radiolysis products formed in irradiated polymers, will be presented. The evaluation of new packaging materials for irradiated foods will be discussed within the context of FDA's Food Contact Substance Notification Program.

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In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

In the 1960s, the FDA approved many packaging materials for use during the irradiation of prepackaged food with only two additional materials receiving approval since (see below for details). Most of the approvals were obtained by the U.S. Army and the U.S. Atomic Energy Commission (AEC) (*1*). These agencies shared responsibilities under the U.S. Government's Atoms for Peace program to develop peaceful uses for nuclear technology. The U.S. Army was particularly interested in radiation-sterilization to add to the arsenal of methods for producing shelf-stable foods for the military (*2, 3*). Why, after 40 years, is there sudden industry interest in obtaining FDA approval for new packaging materials for use during the irradiation of prepackaged food that is intended for consumption by the general public? The answer can be summed up in two words: emerging pathogens.

The following timeline illustrates increasing concern about pathogens and interest in new technologies, such as irradiation, for reducing pathogen levels in meat and poultry:

1982 *E. coli* O157:H7 was first linked to serious illness from eating undercooked meat (*4*).

1990 The FDA approved the irradiation of fresh or frozen uncooked poultry at doses up to 3 kGy in response to food additive petitions (FAP) submitted by the U.S. Department of Agriculture and Radiation Technology, Inc. (*5*).[1] The impetus for these petitions was a heightened awareness of the threat to public health from food-borne illnesses caused by *Salmonella*, *Yersinia*, and *Campylobacter* on poultry.

1993 The widely publicized Jack-in-the-Box incident occurred in which numerous illnesses and four deaths, primarily among children, were caused by *E. coli* O157:H7 present in undercooked hamburgers served at the fast food chain in four states of the Western U.S. (*4, 6*).

1997 In response to a petition submitted by Isomedix, Inc., the FDA approved the irradiation of uncooked meat at doses up to 4.5 kGy for refrigerated products and up to 7.0 kGy for frozen products (*7*).

2001 In response to a petition submitted by the National Center for Food Safety and Technology, Illinois Institute of Technology (NCFST), the FDA deemed that the three radiation sources permitted for use on food, gamma, X-ray, and e-beam, are equivalent in terms of the types and levels of radiolysis products (RP) generated in the packaging materials under the conditions at which prepackaged foods are irradiated (*8*). This decision expanded the combinations of packaging materials and radiation sources that may be used on food.

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[1] The Gray (Gy) is a unit of radiation-absorbed dose that equals the amount of energy absorbed per unit mass of a material during irradiation (1 Joule/kg). 10 kGy = 1 Megarad (Mrad), a previous unit of absorbed dose.

The list of FDA-approved materials does not adequately cover the expansive number of polymers, adhesives, and colorants that are used in multi-layer, multi-constituent food-packaging materials that offer special properties such as an improved oxygen barrier. In addition, very few of the adjuvants (e.g., antioxidants, plasticizers, and antifogging agents) that are routinely used in today's materials have been evaluated and approved by the FDA for use in packaging materials that are intended to be irradiated in contact with food.

Legal Considerations: Why Is FDA Approval Necessary?

Background

The *Federal Food, Drug, and Cosmetic Act* (or the "*Act*"), Section 409(a), states that the use of a food additive shall conform to a regulation prescribing the conditions under which the additive may safely be used. Section 201(s) of the *Act* defines a food additive, in part, as "any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of food." The definition encompasses packaging because packaging components could become a component of food by migrating from the packaging into food. In the past, packaging components have been referred to as "indirect additives" because these substances are not added directly to food for some functional purpose. Section 201(s) also defines any source of radiation intended for use on food as a food additive. Under Section 409 of the *Act*, as originally established, food additives require premarket approval by the FDA through the submission of a food additive petition and publication of a regulation authorizing their intended use. The requirements for a petition are described in Title 21 of the *Code of Federal Regulations* (*CFR*), Part 171.1 (Petitions) (*9*). FDA's safety evaluation of a food additive includes a dietary exposure assessment and a toxicological evaluation based on animal feeding studies and other toxicological information.

Recently, Section 309 of the FDA Modernization Act of 1997 (FDAMA) amended Section 409 of the *Act* to establish a new process, referred to as the food contact notification (FCN) process, as the primary method of authorizing new uses of food additives that are food contact substances (FCS). Section 409(h)(6) defines an FCS as "any substance intended for use as a component of materials used in manufacturing, packing, packaging, transporting, or holding food if such use is not intended to have any technical effect in such food." The requirements for an FCN are described in 21 *CFR* 170, Subpart D (Food Additives – Premarket Notifications) (*9*), and guidance documents are available on FDA's website (*10*). Because the safety standard is the same for all food additives, the data and information requirements for FCNs and petitions are comparable.

There are two main differences in the petition and FCN processes. First, in contrast to the petition process, the FCN process will not result in a food additive listing in the *CFR* authorizing the use for any manufacturer of the FCS. Rather, an FCN for a food contact substance cannot be effective for anyone other than the manufacturer identified in the FCN. FDA maintains a list of effective FCNs on its website (*10*). Second, under the FCN process, the FDA has 120 days in which to object to an FCN or the FCN becomes effective, and the FCS may be legally marketed for the intended use.

Irradiated Packaging

A reference list of materials currently permitted for use during the irradiation of prepackaged food is given in Table I. With the exception of polystyrene (PS) foam trays, all the materials in Table I are listed in 21 *CFR* 179.45 (Packaging materials for use during the irradiation of prepackaged food) (*9*). The PS foam trays were reviewed under FDA's Threshold of Regulation policy, which exempts certain food additives from a food additive regulation listing when the use results in a dietary concentration (DC) of less than 0.5 ppb (see 21 *CFR* 170.39 (*9*)).

In addition to §179.45, two other sections of the *CFR* are applicable to the irradiation of prepackaged food: §179.26 (Ionizing radiation for the treatment of food) and §179.25(c) (General provisions for food irradiation). Section 179.26 lists the radiation sources that may be used on food, the specific foods that may be irradiated, the conditions under which those foods may be irradiated, and the labeling that is required on irradiated foods. Section 179.25(c) inextricably links the packaging materials listed in §179.45 with the conditions of use described in §179.26, meaning that no other packaging materials (including adjuvants) are permitted for prepackaging food that will be irradiated. The finished packaging material and all adjuvants must meet any specifications and limitations of the applicable regulations in order to be marketed in the U.S. for food contact.

Although the vast majority of food contact substances are evaluated via the FCN process, it is still possible that an FCS might require evaluation via the petition process, especially if its dietary concentration is exceptionally high (on the order of 1 ppm or higher; see §170.100(c)(1)) (*9*). The FCN process can take much less time than the petition process because of the additional time

Table I. Materials Currently Permitted for Use During Irradiation of Prepackaged Food

Year	*Regulation*	*Material*	*Requester*	*Max. Dose (kGy)*
1964	§179.45(b)	Nitrocellulose-coated cellophane	AEC	10
		Glassine paper	AEC	10
		Wax-coated paperboard	AEC	10
		Polyolefin film[a]	AEC	10
		Polystyrene film[a]	AEC	10
		Rubber hydrochloride film[a]	AEC	10
		Vinylidene chloride-vinyl chloride copolymer film[a]	AEC	10
1965	§179.45(b)	Vinylidene chloride copolymer-coated cellophane	AEC	10
	§179.45(d)	Vegetable parchments	U.S. Army	60
1967	§179.45(b)	Kraft paper to contain only flour	U.S. Army	0.5
	§179.45(d)	Polyethylene film[a]	U.S. Army	60
		Polyethylene terephthalate (PET) film[a]	U.S. Army	60
		Nylon 6 film[a]	U.S. Army	60
		Vinyl chloride-vinyl acetate copolymer film[a]	U.S. Army	60
1968	§179.45(b)	Optional adjuvants for polyolefin films plus optional vinylidene chloride copolymer coating	AEC	10
		PET film plus optional adjuvants, vinylidene chloride copolymer and polyethylene coatings	AEC	10
		Nylon 11	AEC	10
1989	§179.45(c)	Ethylene-vinyl acetate copolymers	Cryovac	30
1996	Threshold of Regulation submission	Polystyrene foam tray	Amoco	7.2

[a] Plus limited optional adjuvants.

needed to prepare and publish a regulation in response to a petition. The Threshold of Regulation policy (described above) is another route to FDA approval of new FCSs.

Exposure to Radiolysis Products from Currently Regulated Packaging Materials

In order to approve NCFST's petition regarding the equivalency of the three radiation sources that may be used on prepackaged food (see the timeline above), it was necessary to reevaluate the dietary exposure to RPs formed in the packaging materials currently listed in §179.45 for the following reasons:

- Approximately 40 years had passed since FDA first evaluated the materials.
- Several modern analytical methods, e.g., gas chromatography (GC) with mass spectrometric detection, became commercially available in the early 1970s, making it possible for the first time to obtain rapid, quantitative results for numerous individual organic chemicals (*11*). These types of data, particularly for volatile RPs, were not available when FDA first evaluated the materials.
- Over the years, FDA has developed new methods for calculating dietary exposure to FCSs that incorporate marketing data for specific types of food-packaging materials and the types of food that are packaged in them (*12*). Exposure estimates based on consumption factors (CF), which are the fraction of all food in the daily diet that contacts a particular type of packaging material, and food-type distribution factors (f_T), which are the fraction of food packaged in a particular packaging material that is aqueous, acidic, alcoholic, or fatty, are more realistic than those generated in the 1960s.
- It was necessary to determine if there would be any increase in dietary exposure to RPs formed in the packaging materials if all three radiation sources could be used on all the packaging materials currently listed in §179.45.

Relevant Parameters

The first step in evaluating exposure to RPs from packaging materials was to identify and quantify parameters that depict the conditions under which prepackaged food would be irradiated and stored. Based on an extensive review of the literature, the following six parameters were determined to be relevant to RP formation in polymers:

Absorbed Dose

Irradiation leads to two competing reactions in polymers: chain scission, which leads to the formation of low-molecular-weight RPs, and crosslinking, which can lead to a decrease in residual oligomers (*13, 14, 15*). An increasing absorbed dose can lead to crosslinking up to an optimum point. If the dose is increased beyond that point, chain scission becomes dominant. In the absence of crosslinking, which occurs only in an O_2-free atmosphere (see below), concentrations of RPs generally increase linearly with absorbed dose within limited dose ranges that include the ranges needed for irradiating foods (*13, 14, 15, 16*).

Because fresh or frozen poultry, fresh meat, and frozen meat may be irradiated to doses up to 3, 4.5, and 7.0 kGy, respectively, and because only a few foods of limited consumption may be irradiated to higher doses,[2] 10 kGy was selected as a conservative value for use in exposure estimates for polymer RPs that form in foods irradiated in their final packaging.

Atmosphere

In the presence of oxygen or air (21% O_2), polymer chain scission leads to the formation of oxidative degradation products, which are primarily oxygenated volatile and semi-volatile organic compounds such as aldehydes, ketones, and carboxylic acids (*13, 15, 17, 18, 19, 20, 21, 22*). Crosslinking dominates under vacuum or an inert atmosphere. In air, increasing dose leads to higher concentrations and a wider variety of measurable RPs (*21*). For example, for low-density polyethylene (LDPE) irradiated to 20 kGy with an e-beam source at room temperature, the levels of oxygenated volatile and semi-volatile organic compounds are about one order of magnitude higher in polymer samples irradiated in air than in those irradiated in a vacuum, while the levels of hydrocarbons are the same in the presence or absence of O_2 (*17*).

In the U.S., all commercial facilities that irradiate food and other bulk materials such as medical supplies are currently irradiating in air (*23, 24, 25*). If the food is packaged in a material that contains an oxygen barrier and the interior is purged of oxygen, then oxygenated RPs are not likely to form in the packaging layers inside that barrier or in the food. However, if the interior is not completely purged of oxygen, RPs may form in the inner layers of the packaging material and migrate to the food.

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[2] Dry enzyme preparations may be irradiated to 10 kGy, dry spices to 30 kGy, and frozen, packaged meats used solely in NASA space flight programs to 44 kGy (see §179.26).

Dose Rate

In the presence of air, for a given dose, the low dose rates typical for gamma sources can lead to levels of RPs in polymers that are higher than levels generated at the higher dose rates typical for X-ray and e-beam sources;[3] the latter, for a given dose, also result in the formation of fewer types of detected RPs (*13, 15, 17, 19, 20, 22, 26*). Exposure data based on studies of migrants from polymers irradiated by gamma sources can therefore be considered conservative for migrants formed by any source of radiation.

The difference between the levels of RPs generated by gamma and e-beam sources is generally not great at doses below 20 kGy (*20*). For LDPE irradiated to 20 kGy in air at room temperature, the levels of RPs induced by gamma radiation exceed those induced by e-beam radiation only by about a factor of two (*17*). Therefore, when gamma irradiation data are unavailable, it is reasonable to use the levels of RPs generated by e-beam or X-ray sources to estimate exposures at low doses (<20 kGy), particularly considering the uncertainties involved in the exposure calculations (see below).

Temperature

In general, the temperature of a polymer during irradiation does not have an effect on RP concentrations when the polymer is irradiated below its glass transition temperature (T_g). But, as the temperature is increased above the T_g, the concentrations of RPs can increase significantly (*17*). For LDPE irradiated to 20 kGy with an e-beam source in air at room temperature, the concentrations of volatile and semi-volatile RPs have been shown to increase by about a factor of three between -75° C (T_g = -78° C) and 0° C, at which point they level off (*17*).

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[3] Due to the lower efficiency of machines in generating X-radiation compared to e-beam radiation and due to the large mass of material that would be required to absorb the more penetrating X-rays, the dose rate for X-ray sources is lower than that for e-beam sources. The sources of radiation, listed in order of increasing dose rate, are: gamma << X-ray < e-beam.

Because fresh or frozen poultry and meat are expected to contribute significantly to the total daily diet among irradiated prepackaged foods, the FDA has generally assumed that half of all foods irradiated in their final packaging will be treated at a temperature ≤ 4° C (fresh) and the other half at -18° C or below (frozen), the temperatures recommended in ASTM Standard F1356 for irradiation of poultry and meat (*27*).

Time after Irradiation

After a polymer has been irradiated in air, RP concentrations increase for some time and then level off. This behavior indicates that radiation-induced peroxy radicals become trapped in the polymer, where they continue to react with the polymer and generate RPs until they have all reacted (*16, 20, 28*). For polypropylene (PP) irradiated to 10 kGy with an e-beam source in air at room temperature, the concentrations of volatile (low molecular weight) RPs formed primarily from the polymer have been shown to level off after about 15 days, while the concentrations of less volatile (higher molecular weight) degradation products of Irganox and Irgafos antioxidants used in the polymer can increase by a factor of 2 to 5 during a period of 1 to 60 days after irradiation, at which point they level off (*16, 28*). These facts indicate that, after irradiation, PP degradation reaches a steady state more rapidly than antioxidant degradation.

Exposure estimates for species that migrate from packaging to food are based on the concentrations of migrants (e.g., RPs) in food or food simulants determined under time and temperature conditions that reflect processing and extended storage prior to consumption (*12*). In the absence of market data, it is not possible to estimate how much irradiated poultry or meat is consumed immediately after purchase and how much is stored by consumers in their freezers for some time. Therefore, the FDA has assumed, for the purposes of this exposure evaluation, that fresh poultry and meat are maintained at 4° C for 3 days after irradiation, frozen at -18° C for 6 months (180 days), and then thawed at 4° C for 1 day prior to preparation and consumption. The FDA has also assumed that frozen poultry and meat are maintained at -18° C for 6 months and then thawed in the packaging at 4° C for 1 day. These assumptions should not lead to overly exaggerated exposure estimates because the rate of migration (diffusion) of RPs is greatly reduced at freezing temperatures compared to the rate at room temperature.

Contact with Food Simulants

A "realistic" testing scenario for determining the concentrations of polymer RPs in food would involve irradiating the polymer while it is in contact with a food or food simulant and then conducting a migration study on the same irradiated sample. However, from the point of view of analyzing the RPs and distinguishing those generated in the polymer from those generated in the food simulant, a more "practical" approach involves irradiating the polymer alone, analyzing the polymer for RPs, and calculating exposure estimates using migration modeling or the assumption of 100% migration to food (*12*). These results can be further refined by irradiating a second sample of the polymer alone and then placing it in contact with an appropriate food simulant in order to conduct a migration study using the appropriate testing protocols. The food simulant could then be analyzed for the RPs already identified in the first polymer sample.

One study, which involved aspects of both the realistic and practical testing scenarios, reported that the concentrations of RPs produced in polymers differ by at most a factor of 2 in polymers irradiated in contact with air on one side and with aqueous/acidic food simulants on the other side, compared with polymers irradiated in air alone (*16*).[4] The somewhat higher concentrations in polymers irradiated in contact with food simulants are likely due to the direct contact of the polymer with liquids comprised of oxygenated species. For example, the concentration of acetone in PP film was 2.6 mg/kg when the polymer was irradiated in air alone, 3.7 mg/kg when the polymer was irradiated in contact with water, 2.9 mg/kg when the polymer was irradiated in contact with 15% ethanol, and 4.2 mg/kg when the polymer was irradiated in contact with 3% acetic acid (*16*).[5]

Based on these results, it may be concluded that RP migration values derived from the "practical" scenario are not likely to yield significantly different exposure estimates from those derived from the "realistic" scenario, particularly considering the conservatisms already built into the exposure estimates (see below).

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[4] In these experiments, the RPs were analyzed in the polymer, not the food simulant.

[5] These values were extrapolated from 50 kGy to 10 kGy. The article did not provide data to show whether any of the reported differences among the tested samples were statistically significant.

Exposure Evaluation

Based on a comprehensive collection of recent literature, several organic RPs were identified and quantified in seven of the major polymers listed in §179.45, which made it possible to calculate exposures to the RPs using the relevant parameters described above. The polymers, RPs, and dietary concentrations (DC) are summarized in Table II. In general, the test polymers had been irradiated to 10-50 kGy with gamma or e-beam sources in air at room temperature in the absence of food simulants, and the polymers were analyzed within one day of irradiation. Concentrations of RPs obtained at 20-50 kGy were extrapolated to 10 kGy, assuming a linear relationship between concentration and dose (see the "Absorbed Dose" section above).

Because RPs are expected constituents of packaging resulting from its conditions of use, the DCs were compared to 0.5 ppb, the DC that FDA equates to negligible risk for a substance that has not been shown to be a carcinogen in humans or animals and for which there is no reason, based on the chemical structure of the substance, to suspect that it is a carcinogen. It should not be construed that substances whose DCs exceed 0.5 ppb are unsafe nor that substances of DC ≤ 0.5 ppb are exempt from FDA approval. All food additives, including their related constituents, must be evaluated by the FDA for safety on a case-by-case basis, regardless of their exposures, and may be deemed safe at DCs well above 0.5 ppb.

Calculation Methods

Because the RPs were analyzed in the polymers rather than in food simulants, DCs were initially calculated by assuming 100% migration of the RPs to food. The following parameters were used in this calculation: a polymer film thickness of 40 μm (0.004 cm, 1.57 mils), a density typical of each polymer (see Table II), a food mass-to-polymer surface-area ratio of 10 g/in^2 (1.55 g/cm^2), and a CF of 0.05. FDA's default CF of 0.05 was used because 1) only polymer films are regulated in §179.45 – the CFs given in (*12*) for the various polymers do not distinguish between rigid containers and films, and 2) only a small fraction of food packaged in contact with a given polymer is expected to be irradiated. For PP and Nylon, the CFs given in (*12*) were used (0.04 and 0.02, respectively). Because the packaging materials listed in §179.45 are not restricted by food

Table II. Exposures to Radiolysis Products from Polymers Irradiated to 10 kGy (DCs in Bold Exceed 0.5 ppb)

Polymer/RP	*Conc. in Polymer (mg/kg)*[a, b]	*Ref.*	*Conc. in food (μg/kg)*[c]	*DC (ppb)*
Polystyrene (PS) (density 1.06 g/cm^3)				
1-phenylethanol	3	(*29*)	8.2	0.41[d]
acetophenone	18	(*29*)	fresh: 7.8	0.39[e]
benzene	1	(*29*)	2.7 fresh: 0.53 froz.: 0.36	0.14[d] 0.02[e]
phenylacetaldehyde	3	(*29*)	8.2	0.41[d]
benzaldehyde	18	(*29*)	fresh: 8.4	0.42[e]
phenol	5	(*29*)	fresh: 2.5	0.12[e]
benzoic acid	4	(*29*)	fresh: 1.7	0.09[e]
unidentified carboxylic acid a	2.7	(*30*)	7.4	0.37[d]
unidentified carboxylic acid b	2.7	(*30*)	7.4	0.37[d]
Poly(ethylene terephthalate) (PET) (density 1.4 g/cm^3)				
diisopropyl ether	0.8	(*30*)	2.89 fresh: 0.11	0.14[d] 0.006[e]
formic acid	0.297	(*31*)	1.0	0.05[d]
acetic acid	0.369	(*31*)	1.3	0.06[d]
1,3-dioxolane	0.384	(*31*)	1.4	0.07[d]
2-methyl-1,3-dioxolane	3.7	(*31*)	fresh: 0.55	0.03[e]
acetone	0.086	(*31*)	0.30	0.02[d]
Low-Density Polyethylene (LDPE) (density 0.92 g/cm^3)				
acetic acid	8.5	(*17*)	8.5	**1.0**[d, f]
propionic acid	5.1	(*17*)	12	**0.6**[d, f]
n-butyric acid	1.0	(*17*)	2.4	0.12[d]
n-valeric acid	0.4	(*17*)	0.95	0.05[d]
butanoic acid vinylester or 2-furanmethanol	1.68	(*30*)	4.0	0.20[d]
1,3-di-*tert*-butylbenzene from Irgafos 168	1.7	(*30*)	4.0	0.20[d]
2,4-di-*tert*-butylphenol from Irgafos 168	30	(*32*)	71	**3.6**[d, f]
2,6-di-*tert*-butyl-*p*-benzoquinone from Irganox 1010, 1076	4	(*32*)	9.5	0.47[d]

Table II. Exposures to Radiolysis Products from Polymers Irradiated to 10 kGy (DCs in Bold Exceed 0.5 ppb), contd.

Polymer/RP	*Conc. in Polymer (mg/kg)*[a, b]	*Ref.*	*Conc. in food (µg/kg)*[c]	*DC (ppb)*
Polypropylene (PP) (density 0.90 g/cm^3)				
2,4-pentanedione	2.4	(*16*)	5.6	0.22[d]
1-dodecene	1.4	(*16*)	1.4	0.13[d]
acetone	2.6	(*16*)	6.0	0.24[d]
2-pentanone	0.75	(*16*)	1.7	0.07[d]
4-hydroxy-4-methyl-2-pentanol (?)	1.9	(*30*)	4.4	0.18[d]
3-methyl-2-butanone (?)	1.5	(*30*)	3.5	0.14[d]
acetic anhydride	7.4	(*30*)	17	**0.69**[d, f]
3-methylbutanoic acid	2.0	(*30*)	4.6	0.19[d]
acetic acid-(1-ethylhexyl)-ester	0.7	(*30*)	1.6	0.07[d]
octanoic acid	1.8	(*30*)	4.2	0.17[d]
3-methyl-4-methylene-hexane-2-one	0.9	(*30*)	2.1	0.08[d]
2,5-cyclohexadiene-1,4-dione	2.1	(*30*)	4.9	0.20[d]
hexadecanol or octadecanol	2.0	(*30*)	4.6	0.19[d]
4-methyl-2,3-pentanedione (?)	1.1	(*30*)	2.6	0.10[d]
1,3-di-*tert*-butylbenzene from Irgafos 168	17	(*16*)	39	**1.6**[d, f]
2,4-di-*tert*-butylphenol from Irgafos 168	75	(*32*)	174	**7.0**[d, f]
	16[g]	(*33*)	28	**1.1**[h]
1,3-di-*tert*-butyl-2-hydroxybenzene from Irgafos 168	14	(*16*)	33	**1.3**[d, i]
2,6-di-*tert*-butyl-*p*-benzoquinone from Irganox 1010, 1076	14	(*28*)	33	**1.3**[d, i]
Ethylene-Vinyl Acetate Copolymers (EVA) (density 0.94 g/cm^3)				
acetaldehyde	--	(*34*)	1600	**32**[j]
n-propyl acetate	--	(*34*)	570	**11**[j]
3-methylhexane	--	(*34*)	1000	**20**[j]
n-heptane	--	(*34*)	430	**8.6**[j]
n-octane	--	(*34*)	67	**1.3**[j]
Nylon 6 (density 1.1 g/cm^3)				
butanamide	2	(*35*)	5.7	0.11[d]
pentanamide	85	(*35*)	fresh: 42 froz.: 29	**0.71**[e]

Table II. Exposures to Radiolysis Products from Polymers Irradiated to 10 kGy (DCs in Bold Exceed 0.5 ppb), contd.

Polymer/RP	*Conc. in Polymer (mg/kg)*[a, b]	*Ref.*	*Conc. in food (μg/kg)*[c]	*DC (ppb)*
Poly(vinyl chloride) (PVC) (density 1.3 g/cm^3)				
4-hydroxy-4-methyl-2-pentanone	6.2	(*30*)	21	**1.0**[d, i]
5-hexen-2-one	3.8	(*30*)	13	**0.64**[d, i]
1-ethoxy-2-heptanone	7.1	(*30*)	24	**1.2**[d, i]
methoxyacetaldehyde diethyl acetal	15	(*30*)	50	**2.5**[d, i]
diethoxy acetic acid ethylester	4	(*30*)	13	**0.67**[d, i]
3-methylheptyl acetate	2.4	(*30*)	8.0	0.40[d]
diethyl adipate	8.3	(*30*)	28	**1.4**[d, i]
nonanoic acid ethylester	2.4	(*30*)	8.0	0.40[d]
unidentified *n*-alkane acid ethylester a	34.5	(*30*)	116	**5.8**[d, i]
unidentified *n*-alkane acid ethylester b	50.3	(*30*)	169	**8.4**[d, i]

[a] Concentrations determined at 20-50 kGy were extrapolated to 10 kGy, assuming a linear relationship between concentration and dose. Concentrations reported for unirradiated control samples were subtracted from those reported for irradiated test samples.

[b] Only the highest concentration reported for each RP in the literature is included in this table.

[c] Assuming a food mass-to-polymer surface area ratio of 10 g/in^2 (see text).

[d] 100% migration calculation.

[e] Modeled migration (see text).

[f] Migration models failed to describe migration below 100% from thin films made of polymers that yield fast diffusion coefficients.

[g] Migration to 10% ethanol food simulant expressed as mg/kg polymer tested.

[h] Measured migration value into 10% ethanol after 10 d at 40° C.

[i] Migration modeling not possible due to lack of diffusion coefficients for PVC films.

[j] Measured migration value into 95% ethanol after 1 d at room temperature.

type, a total f_T value of 1 was assumed. A sample calculation follows for a level of 3 mg/kg 1-phenylethanol (PhE) in PS film (density: 1.06 g/cm^3, thickness: 40 μm or 0.004 cm):

$$\left(\frac{3\times10^{-6}\ \mathrm{g\ PhE}}{\mathrm{g\ PS}}\right)\left(\frac{1.06\ \mathrm{g\ PS}}{\mathrm{cm}^3}\right)\left(\frac{0.004\ \mathrm{cm}}{}\right)\left(\frac{6.45\ \mathrm{cm}^2}{1\ \mathrm{in}^2}\right)\left(\frac{1\ \mathrm{in}^2}{10\ \mathrm{g\ food}}\right)\left(\frac{0.05\ \mathrm{CF}}{}\right)=0.41\ \mathrm{ppb\ DC}$$

For cases in which the DCs from 100% migration calculations exceeded 0.5 ppb, migration modeling based on Fick's law of diffusion was used to calculate a more realistic exposure (*36*). The Piringer model was used to calculate diffusion coefficients for use in the migration model (*37*). The time and temperature conditions for fresh poultry and meat described in the "Time after Irradiation" section above were used as inputs for the model. Then, to calculate the DC, the food mass-to-polymer surface-area ratio, CFs, and f_T described above were applied to the modeled migration values.

If the DC exceeded 0.5 ppb for fresh poultry and meat, the FDA assumed that half the products will be irradiated fresh and that half will be irradiated frozen (see the "Temperature" section above). This assumption led to two terms in the DC calculation, each of which was dominated by the 6 months at -18° C time and temperature condition. Because the two terms were practically equal, this calculation did not reduce any exposure estimates for fresh products to ≤ 0.5 ppb DC. Nevertheless, this calculation did yield the most realistic exposure estimates possible for the RPs (see, for example, the Polystyrene and Nylon 6 entries in Table II).

Summary of Results

Exposure information from Table II on the 58 RPs quantified in seven major polymers is summarized in Table III. From this table, it is evident that the majority of the RPs (31) met the 0.5 ppb DC limit based on a 100% migration calculation and that five more met the limit based on migration modeling. For the remaining 22 RPs that did not meet the 0.5 ppb DC limit, it should be noted that the 10-kGy dose used in the exposure estimates is conservative because fresh or frozen poultry and meat, which may be irradiated to 3 to 7 kGy, are expected to contribute significantly to the total daily diet among irradiated prepackaged foods. In addition, the test polymers had been irradiated at room temperature. If the polyolefins (T_g << 20° C) had been irradiated at the intended refrigerated or frozen use temperatures, the DCs of their RPs likely would have been lower (see the "Temperature" section above).

Table III. Results from Comparing Radiolysis Product Exposures to 0.5 ppb DC

Polymer	*Number of Radiolysis Products*			
	DC ≤ 0.5 ppb		*DC > 0.5 ppb*	*Total Quantified*
	100% Migration	*Modeled*		
PS	5	4		9
PET	5	1		6
LDPE	5		3	8
PP	13		5	18
EVA copolymers			5	5
Nylon 6	1		1	2
PVC	2		8	10
Total:	31	5	22	58

It should be noted that, although none of the exposures to RPs from PET exceeded 0.5 ppb DC, five of the RPs were determined in test samples that had been irradiated in the absence of O_2 (*31*), which could result in underestimates of exposure if food packaged in PET film were irradiated without an oxygen barrier between the PET and the ambient air of the radiation chamber (see the "Atmosphere" section above). It is not likely that the presence of O_2 would cause the exposures to the identified RPs to exceed 0.5 ppb DC because 1) exposure estimates based on an absorbed dose of 10 kGy are conservative for foods that are irradiated in their final packaging, and 2) the five exposure estimates are approximately one order of magnitude below 0.5 ppb DC (see Table II). Only through further testing would it be possible to identify and quantify additional RPs that might form when PET is irradiated in the presence of O_2.

Radiolysis Products Whose Exposures Exceeded 0.5 ppb DC

Polymer Adjuvants

Thirteen of the 22 RPs with DCs > 0.5 ppb were from polymer adjuvants that are not listed in §179.45. 2,4-Di-*tert*-butylphenol (2,4-DTBP), which was identified in irradiated LDPE and PP, is a breakdown product of Irgafos 168, an antioxidant often added to polyolefins (*32*). Three additional breakdown products of Irgafos 168 and a breakdown product of the antioxidants Irganox

1010 and 1076 were also identified in PP (see Table II). These breakdown products also form during photochemical and thermal oxidation of packaging materials containing hindered phenol antioxidants (*28*). However, the kinetics of their formation is much more rapid during irradiation, i.e., irradiation is comparable to accelerated ageing (*28*). The FDA has typically not been concerned with the breakdown products of these antioxidants because they have not been observed under conventional food-contact conditions.

Although basic polyolefins listed in §177.1520 (Olefin polymers) are regulated for use as films under §179.45(b)(4) and §179.45(d)(2)(i), the optional adjuvants listed in §177.1520(b) and the antioxidants and stabilizers listed in subparagraph (b) of §178.2010 (Antioxidants and/or stabilizers for polymers) such as Irgafos 168, Irganox 1010, and Irganox 1076 are not (*9*). Therefore, polyolefins containing the adjuvants listed in §177.1520(b) or §178.2010(b) are not currently permitted for prepackaging food that will be irradiated and would need to be evaluated for safety via FDA's food contact notification process.

In addition to the exposure calculated assuming 100% migration of 2,4-DTBP from irradiated PP to food (7.0 ppb DC), actual migration data in the literature made it possible to obtain a more realistic exposure (*33*). In the study, a 10% ethanol food simulant was sealed inside a PP pouch and irradiated to 10 kGy with an e-beam source in air at room temperature. The pouch was then maintained at 40° C for 10 days prior to analysis. The resulting DC (1.1 ppb) is about a factor of 6 less than the 100% migration calculation noted above.

All eight of the RPs from PVC whose DCs exceeded 0.5 ppb are RPs of the plasticizer (probably di(2-ethylhexyl)adipate, based on the presence of diethyl adipate) rather than of the PVC itself. PVC is regulated only as an optional adjuvant for polyolefin films and PET films in §179.45. PVC films *per se* are not currently permitted for prepackaging food that will be irradiated. Therefore, the likely exposure to the RPs from regulated plasticized PVC will be well below 0.5 ppb DC.

EVA Copolymers

The migration data for the five RPs from EVA copolymers whose DCs exceed 0.5 ppb are from Food Additive Petition 7B3968, which resulted in the listing of EVA copolymers in §179.45 (*34*). EVA copolymer pouches were filled with 95% ethanol food simulant and irradiated to 30 kGy with a gamma source in air at room temperature. The food simulants were maintained at room temperature until they were analyzed one day after irradiation. In order to calculate the DC, migration values were extrapolated to 10 kGy, a food mass-to-polymer surface area of 10 g/in^2 was assumed, and a CF of 0.02 (the CF for EVA copolymers (*12*)) was applied. These values are slightly lower than the exposures originally calculated for the petition, which were based on the 30-kGy dose and an additional assumption that 40% of food packaged in EVA copolymers would be irradiated. Although the DCs for these RPs exceed 0.5 ppb DC, they were individually evaluated by the FDA and determined to be safe.

GRAS Substances

Acetic acid and propionic acid are two RPs from LDPE. These two acids are affirmed as Generally Recognized as Safe (GRAS) for direct addition to food in §184.1005 and §184.1081, respectively. Thus, they have been deemed safe at DCs much greater than 0.5 ppb (*9*). Both acids can have a significant effect on the organoleptic properties of food, so their concentrations are self-limiting at levels far below their good manufacturing practice use levels described in the regulations.

The acetic anhydride RP from PP is likely to hydrolyze to acetic acid in food. In addition, it was necessary to use a 100% migration calculation for acetic anhydride because the simple migration model, coupled with the Piringer model for calculating diffusion coefficients, indicates that 100% migration will occur from thin PP films. Simple migration models assume that an infinitely thick plane of material is in contact with the food (i.e., an infinite source of the migrant) and therefore do not depend on the polymer thickness (*36, 37*). The models generally fail in describing migration below 100% from thin films made of polymers that yield fast diffusion coefficients (e.g., polyolefins). However, the models successfully predicted migration from films made with polymers that yield very slow diffusion coefficients (e.g., PS, Nylon, and PET).

Pentanamide from Nylon 6

The DC of pentanamide from Nylon 6 was calculated via migration modeling to be 0.7 ppb, which exceeds 0.5 ppb by a very small amount. As is discussed above, the 10-kGy dose selected for this exposure evaluation is conservative for foods that are irradiated in their final packaging.

Summary

Exposures to 58 RPs from seven polymer types have been evaluated, based on a survey of the available literature. Many more RPs have been identified in five of the polymer types but have not been quantified (up to 63 in LDPE (*21, 38, 39*), 73 in PP (*21, 39*), 14 in plasticized PVC (*40*), 10 in Nylon 6 (*35*), and

10 in EVA copolymers (*34*)). However, the compounds that have been quantified tended to have the highest GC peak areas, i.e., the highest concentrations in the polymer. Because the RPs are primarily aldehydes, ketones, and carboxylic acids, it can be assumed that the GC response factors for the quantified and unquantified compounds are similar. Therefore, in most instances, the concentrations of the unquantified compounds should not exceed those of the quantified compounds. Quantitative data on RPs formed in the remaining materials listed in §179.45 are not available. However, fresh and frozen poultry and meat are most likely to be prepackaged in the polymeric films discussed above (except plasticized PVC) or multilaminates comprised of them.

Conclusion

Based on a comprehensive review of recent literature articles in which RPs were quantified in major polymers, it has been determined that irradiation of the most commonly used materials listed in §179.45, under the conditions typical for foods, results in exposures to many RPs that are below 0.5 ppb DC. These RPs and others with higher exposures have been evaluated and determined to be safe. Although RPs from the currently regulated materials are not of concern, new polymers might yield RPs of toxicological concern. In addition, the literature data have shown that RPs from unregulated polymer adjuvants such as antioxidants and plasticizers are of potential concern due to their high concentrations. Polymeric materials and adjuvants that are not listed in Table I must be approved by the FDA for use during the irradiation of prepackaged food.

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Chapter 13

Fate of Energy Absorbed by Polymers during Irradiation Treatment

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Introduction

E-beams, x-rays, and γ-photons each interact with polymer molecules to eject orbital electrons (*1*). Less than 0.015% of the total incident energy absorbed by the polymer is absorbed in this initial interaction (*2*). The remaining energy transfer occurs through a cascade of secondary interactions produced when a spray of high-energy ejected electrons produce many subsequent ionizations. Equation (1) illustrates this general process.

$$AB + \text{initial irradiation event} \rightarrow AB^+ + e^- \qquad (1)$$

Inertia propels electrons forward along a certain trajectory. Ionization events occur in tracks and spurs along the path of electron travel (*3*). Ionization does not discriminate between one atomic center and another. Ionization occurs wherever an accelerated electron of sufficient energy passes close enough to polymer electrons that coulombic forces repel the electron with ionizing force (*4*). Under high vacuum conditions characteristic of mass spectroscopy, positively charged parent ions, ion fragments and molecular rearrangements arise through the process:

$$AB^+ \rightarrow A^+ + B \qquad (2)$$

Where B is most often a free radical. Similar initial processes occur in ambient pressure systems, however, wherein complex clustering of proximal molecules produces an array of secondary ion fates that are difficult to predict.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

In gases, where the number of ionizations can be measured directly, the amount of energy absorbed per ion-pair formation is well documented. Table I lists energies required for ion-pair production for several atmospheric and low molecular weight organic gases.

Table I. Average Energy Required per Ion-Pair Production (*4*)

Gas	*Energy/ion-pair (eV)*	*Ionization Potential*	*Ratio*
H_2	36.3	15.4	2.35
N_2	34.7	15.6	2.22
O_2	30.9	12.3	2.50
CO_2	32.8	13.8	2.38
CH_4	27.3	13.1	2.09
C_2H_4	26.3	12.2	2.15
C_2H_2	26.1	11.3	2.30

Radiation chemists often assume a 32.5 eV average for ion-pair production. The energy for ion pair formation in condensed media is more difficult to evaluate, but similar values are assumed. It is clear for compounds referenced in Table I that less than half of the energy required for ion-pair production results directly in ionization. Much of the remaining energy goes to sub-ionization processes. Some remains unexploited by any chemical mechanism and ultimately dissipates as heat (*5*). The following discussion tracks the fate of ionization and sub-ionization processes in irradiated polymers in an effort to establish the theoretical ceiling concentration for radiolytic compounds in polymers irradiated up to a dose of 10 kGy.

Ion-pair Formation and Significance

Ion pairs survive in polymers for about 1 x 10^{-13} sec (*6*). This limited lifetime is too brief for a charged molecule to migrate even a full atomic diameter. Therefore, radiation chemistry involving ionized molecules is strictly contained at the point of initial electron contact. This restriction strongly influences the variety and chemical functionality of ionization-mediated species in irradiated polymers. It requires that ionizations and, consequently, the population of radiation-induced polymer variants will be randomly distributed. Since a single polymer macromolecule contain thousands of bonds, each equally susceptible to ionization by the transient high energy field of passing electrons, the number of unique polymer variants directly resulting from ionization would be similar to the number of molecular bonds. However, the precise number depends on properties of the polymer.

An ideal high-density polyethylene (HDPE) would produce the least diversity. HDPE is a linear homopolymer assembled from a symmetrical monomer. The potential number of compounds directly arising from HDPE ionization would be the total number of bonds in the polymer strand divided by 2, since reactions at either methyl hydrogen on a particular carbon atom will result in the same compound, and divided by 2 again since reactions occurring at sites relative to either end of the symmetrical polymer will produce the identical compound.

However this base number of possible species will be augmented by the chemical diversity expressed at a given location following ionization. These include: free radical formation, recombination of positive ions with a low energy electron (without or without secondary reactions), double bond formation, chemical rearrangements, and chain scissions to list a few possibilities. Diversity and abundance will be further influenced by the environmental setting of the polymer with certain additives sacrificially reducing radiolytic abundances and with oxygen increasing the number and variety of radiolytic compounds (*7*). Multiple ionization events on a single polymer (although relatively rare, as will be subsequently shown) would add additional diversity. Add to all this the reality that no HDPE molecule is ideally linear; but contains occasional erratic branching, and it becomes apparent that randomly focused ionization events, even in the simplest polymer, can only result in thousands of unique compounds created at vanishingly low concentrations.

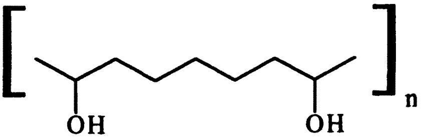
The ionization chemistry of HDPE produces species, which though abundant, are numerically comprehensible. Irradiations of more complex polymers produce species diversity that is perplexing to comprehend. Consider the random interpolation of vinyl alcohol moieties into HDPE so that the resulting polymer is 31 mole percent vinyl alcohol and 69 mole percent ethylene. This formulation represents a commercial grade of a co-polymer of ethylene and vinyl alcohol or EVOH. The analysis which follows examines the upper limit for molar radiolytic abundance due to ionization-mediated chemistry and estimates the number of radiolytic fragments possible from irradiation of EVOH.

Theoretical Ceiling for Compounds Produced Directly By Ionization

If all radiation chemistry resulted directly from reactions between ionized species, it would be an easy matter to calculate the upper bounds for chemical conversions due to irradiation. This value would simply be:

$$\frac{\textit{Total Bonds}}{\left(\dfrac{\textit{Total Deposited Energy}}{\textit{Ion}-\textit{Pair Energy/Bond}}\right)} \qquad (3)$$

The total bonds per Kg of EVOH can be calculated from the effective molecular weight of EVOH subunits. EVOH is a random co-polymer containing ethylene and vinyl alcohol moieties. Since polymerization is random, the pattern of monomer distribution along the chain is diverse and unpredictable. However, statistically, EVOH is composed of 1.63 vinyl alcohol groups per ethylene group with an approximate average formula:



General Formula (4)

Since EVOH is a random co-polymer numerous alternative combinations are possible.

The molecular weight of the ethyl group (CH_2-CH_2) is 28 g/mole while the molecular weight of the vinyl alcohol group (CH_2-CH-OH) is 44 g/mole. The weight-average molecular weight per carbon location on the backbone calculates to:

$$\frac{1.63(14\ \textit{Daltons per} -CH_2- \textit{in vinyl alcohol}) + 1.63(30\ \textit{Daltons per} -CHOH\ \textit{in vinyl alcohol}) + 2(14\ \textit{Daltons per} -CH_2- \textit{in ethylene})}{5.26\ \textit{moles of subunits}} =$$

18.85 g/mole per carbon center. Equation (5) gives the combined moles of subunits in 1 Kg of polymer:

$$\frac{1000g}{18.85 g/mole} = \frac{53.1\ \textit{moles of subunits}}{\textit{Kg polymer}} \qquad (5)$$

Each carbon contains two carbon-hydrogen (or one carbon-hydroxyl and one carbon-hydrogen) bonds and one carbon-carbon bond. The hydroxyl group also contains an oxygen-hydrogen bond. Therefore, on average there are approximately 3.3 bonds per carbon or 175 moles of bonds per Kg of EVOH. Converting moles of bonds to numbers of bonds yields:

$$175\,\mathit{moles\ of\ bonds}\cdot\frac{6.022\cdot 10^{23}\,\mathit{bonds}}{\mathit{mole of\ bonds}} = 1.054\cdot 10^{26}\mathit{bonds} \qquad (6)$$

Absorbed Energy and Bond Conversion at 10 kGy Treatment

The Gray is a measure of absorbed ionizing energy. The amount of energy absorbed from a 10 kGy treatment is:

$$10\ \mathrm{kGy} = \frac{6.25 \times 10^{22}\ \mathrm{eV}}{\mathrm{Kg\ polymer}} \qquad (7)$$

Assuming an average energy for ion-pair formation in organic compounds of 32.5 eV, the number of ion-pairs formed per Kg of polymer would be:

$$\left(\frac{\dfrac{6.25\cdot 10^{22}\,eV}{Kg\ polymer}}{\dfrac{32.5\,eV}{ion-pair}}\right) = 1.92\cdot 10^{21}\,\frac{ion-pairs}{Kg\ polymer} \qquad (8)$$

Therefore, the incidence of ionization would approximately be:

$$\frac{1.054\cdot 10^{26}\,\dfrac{polymer\ bonds}{Kg}}{1.92\cdot 10^{21}\,\dfrac{ion-pairs\ formation}{Kg}} = 54888\,\frac{polymer\ bonds}{ion-pair\ formation} \qquad (9)$$

Thus, only about 1 in 54880 carbons ever experience ionization when irradiated to 10 kGy. This would set something of an upper bound for radiochemical events directly involving ionized species. While EVOH was assumed in the previous calculation, most other polymers would have results in the same neighborhood. It suggests that 1.92 x 10^{21} bonds can be altered by irradiating 1 Kg of EVOH with 10 kGy of ionizing radiation. This would produce 3.2 millimoles of varied radiolytic products per Kg of polymer. A 50 g polymer package could therefore have as much as 160 micromoles of radiolytic products in a package assuming 100% of all energy resulted in bond conversion.

Upper Concentration for Direct Chemical Products of Ionization

On average, each EVOH strand weighs 67,000 Daltons, and each subunit weighs 18.85 Daltons. Therefore, the typical polymer strand contains:

$$\frac{67{,}000\,\frac{Daltons}{strand}}{18.85\,\frac{Daltons}{subunit}} = 3{,}554\,\frac{subunits}{strand} \qquad (10)$$

Since there are 3.3 bonds per carbon, each strand would have:

$$3{,}554\ subunits\ \ x\ \ 3.3\,\frac{bonds}{subunit} = 11{,}728\ bonds/strand \qquad (11)$$

Therefore; the frequency of strand ionization is given by:

$$\frac{54{,}888\,\frac{bonds}{ionization}}{11{,}728\,\frac{bonds}{strand}} = 4.67\,\frac{strands}{ionization} \qquad (12)$$

Thus only 1 in every 4.67 polymer strands will host an ion-pair occurrence. Multiple ionizations on a single strand will occur $(1/4.67)^n$ strands where n is the number of ionization events. A chain scission (i.e. a carbon-carbon bond cleavage) would at most be expected for 1 in every 3.3 events. Since EVOH is a random copolymer with

$$\frac{1.63\,moles\,of\,subunits\,containing{-}OH\ Groups}{5.26\ moles\ of\ subunits\ per\ repeating\ block} = 0.31\ (fraction\ the\ subunits\ contianing\ alcohol\ moeities) \qquad (13)$$

~ 31% (or 1102) of the units composed of -CHOH and ~69% (or 2452) composed of $-CH_2$, the possible combinations (nCr) of subunits for a polymer with 3554 subunits would be:

$$ {}_{3554}C_{(1102)}* = \frac{3554!}{1102!\ 2452!}\cdot \cong 10^{953} \qquad (14)$$

See footnote 1

In actuality, Equation (14) provides a low prediction, since all the variant chemistries possible at a given bond are not considered. For example, this number does not comprehend the difference between a scission, any one of a number of chemical substitutions, an unsaturation, or a crosslink at a given bond position. Neither does it allow for the possibility that two or more radiolytic events will arise on the same polymer strand to create a radiolytic product, which is impossible to create from a one ion-pair per polymer product scenario. Therefore, if the ion-pairs and their subsequent chemistry occur fully at random along the polymer chain, the probability that even two identical molecules could be produced is astronomically low. Of course, hydrogen (or other functional groups which appear at high frequency along the polymer chain) can be produced in abundance by virtue of its frequency and repetition on the polymer chain. This explains in part why hydrogen is usually the most plentiful compound produced while irradiating polymers. Similarly, repetitive functional groups on tertiary and especially quaternary substituted carbons are also produced in high abundance. In the general case for all polymers, the scissions of repeating side-chain functionalities will produce low molecular weight alkanes, acids, and carbonyl-containing compounds of similar carbon number to the side chain. Applying this principal to EVOH, means that scission of -OH groups produces H_2O as a major irradiation product.

It should be apparent from the rarity of ion-pair formation at 10 kGy that exotic compounds cannot be produced in measurable abundance from irradiation of simple polymers. For example, irradiation of EVOH cannot produce measurable levels of benzene. The probability is incomprehensibly low that several ion-pair events would occur simultaneously in time and location on a particular stretch of the polymer chain where benzene formation would be allowable and with all ionization events consummating in such a way that benzene would be produced. Therefore, ionization chemistry must produce rather prosaic compounds within an atomic radius or so of positive ion formation.

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[1] In reality, Equation (14) cannot be evaluated directly by most computers. Stirling's approximation to n! was used where: $n! \approx \sqrt{2\pi n} \cdot n^n \cdot e^{-n}$. Logarithms were used to keep exponents within computer allowances so that log (n!) = ½ log (2n) + ½ log (π) + n log (n) – n log (e), where e is the naperian base 2.71828. To provide scope, the distance between the diameter of an atomic nucleus and the span of the universe is only 37 orders of magnitude. The numbers of possibilities innumerated in Equation (14) are truly astronomical.

Post- Ionization Chemical Events

As Table 1 indicates, less than 50% of the 32.5 eV absorbed in ion-pair formation is actually spent overcoming the ionization potential of covalent bonds. The remaining energy feeds several post-ionization processes. Equation (2) suggests that free radicals are often produced as companion species to ionization. Also, electrons which have either lost too much energy to eject an orbital electron or which pass too far from an orbital electron to repel it with ionizing force can still produce orbital excitations.

$$AB \rightsquigarrow AB^* \qquad (15)$$

where $\rightsquigarrow$ represents a sub-ionizing radiochemical event

These excited species are not unique to ionization. Excitation with light, and thermal processes under certain conditions can produce energetically identical species:

$$AB + h\nu \rightarrow AB^* \qquad (16)$$

$$AB + heat \rightarrow AB^* \qquad (17)$$

Also, electrons, which have spent their kinetic energy through numerous interactions with polymer electrons, will ultimately become excited in the process of charge neutralization:

$$AB^+ + e^- \rightarrow AB^* \qquad (18)$$

The excited complexes, AB*, in Equations (15), (16), (17) and (18) share a variety of quantized states which are indistinguishable with respect to the source of excitation. Therefore, they produce identical secondary reactions. Excited complexes often produce free radicals. There is evidence that this mechanism may predominate in polymers:

$$AB^* \rightarrow A^\bullet + B^\bullet \qquad (19)$$

Free Radicals

Unlike ions, free radicals in amorphous polymers are comparatively long-lived. They may survive for days to months in the glassy and crystalline structures of certain polymers. Free radical survival can produce reaction products at abundances that exceed random statistical probability. Long-term stability allows free radicals the opportunity to migrate or "tunnel" to labile reaction centers at some point remote to their creation. Free radical reactions are favored at (or near) chain ends, at polymer branch points, in the proximity of other free radicals (which produce unsaturation intramolecularly or cross-linking intermolecularly) and at tertiary and quaternary substituted carbons. Also, as with ion-species chemistry, free-radical reactions can occur randomly at any point along the polymer chain.

The translocation of free radicals with subsequent clustering of reactions at various labile centers creates populations of compounds at abundances which far exceed random probability. While irradiation inevitably produces an innumerable array of trace compounds, it also produces a few isolated clusters of compounds derived from chain ends and common labile centers. In EVOH, free radical processes result in bond scissions, crosslinking, double bond formation, water production and production of oxygenated polymer fragments including alcohols, aldehydes, and acids.

Since ions exist for only an exceedingly brief period (1×10^{-13} sec) in the polymer amorphous phase, it has been speculated that free radicals, not ions, are responsible for most of the reactions observed in irradiated packaging polymers. Chapiro (*4*) expressed several lines of evidence which suggest that free radical reactions are the predominant chemical mechanism in many irradiated systems. These include:

Products obtained in the radiolysis of a number of organic compounds are similar to, although not identical with, the products arising from the photolysis of the same compound.

Several classical free radical chain reactions (polymerization of vinyl monomer, chlorination and oxidation of hydrocarbons, decomposition of hydrogen peroxide) have been initiated by ionizing radiation and the kinetics show a great similarity to the corresponding reactions initiated by ultra-violet light or chemical initiators (peroxides or azo-compounds).

Inhibitors for conventional free radical reactions are also effective in many cases when the reaction is initiated by ionizing radiation.

It is generally believed that 40-50% of the incident energy deposited in irradiated polymers results in ionization, a similar amount in primary excitations and as much as 15% in triplet production. Each of these processes may result in immediate chemical reactions, in secondary free radical processes or may post no permanent chemical change. Ultimately, all 32.5 eV ends up as heat or energy stored in such bond conversions as scissions, crosslinkings, desaturations, and (especially in the presence of oxygen) chemical substitutions.

In the experience of our research, irradiation rarely creates novel compound. Instead, compounds produced by irradiation only augment the size of peak areas already present in the gas chromatograms of unirradiated structures. When we observe novel compounds, their molecular weights were typically very low and usually appeared as 2 to 5 carbon linear hydrocarbons, acids, alcohols, and carbonyl-containing compounds. This leaves open the possibility these low molecular weight compounds once existed in the unirradiated polymer but desorbed from the polymer over time. The similarity between polymer radiolytic products and chemical products arising from thermal and UV stresses is impossible to ignore. It appears certain that some common chemical denominator links ionization-related chemical pathways and lower energy pathways involving heat and light.

Conclusion

Ion Chemistry—The ultimate ceiling for ionization-mediated compounds is set by the first law of thermodynamics. One Kg of EVOH irradiated to 10 kGy would create 3.2 millimoles of products assuming all added energy was captured in bond cleavage. Chemistry resulting directly from ion-pair formation is randomly distributed and fixed at the site of ionization. For EVOH and other random copolymers, the vast number of monomer permutations virtually assures that ionization will not produce two of the same molecule. The main exception being when side groups are present at high frequency as with hydrogen and hydroxyl groups in EVOH. To those worried about the safety of ionizing radiation in the food supply with regard to formation of byproducts in the packaging, the complex distribution of primary ionization products should be comforting. It virtually guarantees that any product unique to irradiation will only occur at trifling levels.

Changes in Multiplicity—As with ion chemistry, changes in multiplicity produce highly localized chemistry and are therefore subject to same statistical arguments presented for ion chemistry. Thus, they cannot produce Threshold or Regulation, FDA's 0.5 ppb benchmark separating dietary levels of regulatory concern for non-carcinogenic compounds in food. Below this benchmark compounds are considered to be too dilute to warrant regulatory concern.

Free Radical Chemistry—Free radicals, by virtue of their ability to migrate, can produce chemical outcomes which occur at frequencies greater than predicted by random probability. They are also present in high abundance as offspring of primary ionizations, through direct sub-ionization encounters with high-energy electrons, and as a result of charge neutralization. While many of these conversions produce hydrogen, water, cross-linking and random conversions subject to the statistical limitations attending ionization chemistry;

these conversions produce hydrogen, water, cross-linking and random conversions subject to the statistical limitations attending ionization chemistry; some free radicals will tunnel to the polymer terminus and produce aliphatic, alcohol, carbonyl and acid fragments which generally decrease with carbon number and which are especially prone to scission at hydroxyl-containing carbon centers. It appears free radical mediated compounds are the only species created at levels potentially significant to health. However, these compounds are analogous to compounds created through light and thermal free radical pathways. Therefore, these compounds introduce no novel issues for food safety. It also helps explain why unique chemical markers in irradiated food are difficult to identify.

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October 23, 2009 | http://pubs.acs.org
Publication Date: January 2, 2004 | doi: 10.1021/bk-2004-0875.ch014

Chapter 14

Effects of Ionizing Radiation on Food Contact Materials

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FDA's Office of Food Additive Safety is responsible for the evaluation of food additive petitions and pre-market notifications requesting approval of new food contact materials for irradiation. Our lab supports that review process by conducting research to identify and quantify the products formed in food contact polymers after exposure to ionizing radiation and then determining whether any of these compounds migrate into food. A major objective of this research is to assist in the development of guidance to manufacturers seeking FDA approval to irradiate their products.

Different polyesters, polyamides, a high-density polyethylene, and an ethylene vinyl alcohol copolymer were evaluated before and after exposure to different levels of gamma and electron-beam radiation. Volatile chemicals were isolated from the test materials using static headspace sampling and analyzed by gas chromatography with mass selective detection. Semi-volatile and non-volatile chemicals were extracted from the test materials using a variety of solvents including n-heptane, a fatty food simulating solvent, and the extracts were analyzed using high performance liquid chromatography with mass selective detection. The extracts containing semi-volatiles were also analyzed by gas chromatography with mass selective detection.

Most of the chemicals found are thought to be products of the polymerization process or conversion of the polymer(s) into a finished package, and breakdown products of polymer additives. Although exposure to ionizing radiation increases the concentration of some of these chemicals and decreases the concentration of other chemicals, few of the chemicals determined are thought to be chemicals specifically formed by ionizing radiation. Results of these analyses and data on the migration of some of the chemicals are presented.

The Food and Drug Administration's (FDA) Office of Food Additive Safety is responsible for the evaluation of food additive petitions and pre-market notifications from manufacturers seeking FDA approval of food contact materials and their additives. Our laboratory supports the process by reviewing submitted data, and conducting research to identify and quantify the chemicals that are retained in food contact polymers, and to quantify their migration into foods, if any, and food simulating solvents. These chemicals can be the polymers themselves, residual chemicals from the manufacturing process, additives, contaminants, and alteration products such as degradation products of polymer additives, formed during the manufacture of the packaging or under conditions of use of the food/packaging system, i.e., all environmental conditions from production of the package to the preparation of the food product for consumption. Exposure of a food/packaging system to ionizing radiation is considered a condition of use.

A limited number of packaging films and polymer additives have been approved for the prepackaging of food prior to exposure to ionizing radiation in the presence of oxygen and water vapor (air) (*1*). Vegetable parchments, Kraft paper, and synthetic polymer films such as polyethylene, propylene, styrene, ethylene-vinyl acetate, ethylene-terephthalate and vinylidene chloride copolymers are approved for food contact use during the irradiation of certain foods. FDA approval has been granted for these polymers as needed. For the most part, these food contact materials were approved as flexible containers for the prepackaging of foods prior to their exposure to ionizing radiation. In all of these cases, the food contact interface remained rather dry with limited surface contact with the food, and it was reasonable to expect insignificant migration of packaging components into the food.

Now with the approval of the irradiation of fresh red meat and fresh fruits and vegetables, the lists of previously approved food contact materials and additives have become inadequate (*2 - 4*). These lists are especially limited for fresh meats. Fresh meat is a relatively costly commodity, and is extremely perishable. Fresh meat must be maintained at refrigerated temperatures to gain maximum eye appeal of the consumer. This requires barrier packaging that maintains the proper balance between moisture and oxygen inside the package. Irradiation of meats does increase shelf life, but to gain even greater product shelf life, either newer polymers and additives, or those currently not approved for irradiation will have to be used. There are many polymers with better mechanical and barrier properties than those currently approved for irradiation. Also, there are many new polymer stabilizers and antioxidants that improve the performance of polymers and therefore increase the shelf stability of prepackaged irradiated foods. The problem is that none of these new polymers and additives have been approved for use in packaging intended for exposure to ionizing radiation. The effects of ionizing radiation on all of these polymers and additives must be evaluated. The chemicals formed during irradiation and those levels that migrate into foods must be determined. Validated data must show that the polymers, additives and their by-products are safe.

Polyvinyl chloride homo-polymer (PVC) when plasticized, becomes extremely flexible, soft, has a very high gloss, and can be heat sealed at very low temperatures. It is excellent for the presentation of fresh meats. In fact, it is the major fresh meat wrap used by supermarkets in the United States, but it is not approved for irradiation. There are many other polymers that would be well suited for use in prepackaging meats prior to irradiation. Ethylene-vinyl alcohol copolymers (EVOH) when laminated between other polymers, have extremely high oxygen barrier properties. This would increase the shelf life of meats, but EVOH is not approved for irradiation. Use of adhesives, another group of additives, is severely restricted for use in food contact materials approved for the prepackaging of foods prior to irradiation. Adhesives are needed to bond olefin food contact surfaces or sealant layers to resins such as polyamides, which have exceptional barrier properties, but neither the polyamides nor the adhesives are approved for irradiation.

In this study, different polyesters and polyamides, a food grade high density polyethylene (HDPE) resin, and an ethylene vinyl alcohol copolymer (EVOH) were analyzed before and after exposure to different levels of γ- and e-beam radiation. Irradiation of the polymer specimens was done in the presence of the oxygen available in static air. This permits oxygen to interact with the polymer and its additives during irradiation potentially forming breakdown products that might not be formed if the polymer and additives were irradiated in a controlled, inert atmosphere. Semi-volatile and non-volatile chemicals were extracted from the test materials using a variety of solvents including n-heptane, a fatty food simulating solvent, and the extracts were analyzed using high performance liquid chromatography (HPLC) with mass selective detection (MSD) and Diode Array Detection (DAD). Some of the extracts were also analyzed for semi-volatiles by capillary gas chromatography with mass selective detection (GC-MSD). Volatiles were isolated from the test materials using static headspace sampling and analyzed by capillary gas chromatography with mass selective detection (HS-GC-MSD).

Experimental

Analytes and Standard Solutions

Most chemicals were purchased from Aldrich Chemicals (Milwaukee, WI) and were of the highest quality available for purchase. When possible, standardization by means of isotope dilution was performed with perdeuterated standards such as d-6-benzene, d-4-1, 2-dichloroethane, d-10-ethyl benzene and d-4-*ortho*-dichlorobenzene. Some of the chemicals were research standards supplied by polymer and additive manufacturers. Converters and polymer manufacturers supplied sheets, powders and pellets of various research polymers.

Standards for HPLC-MSD and HPLC-DAD Analyses. Prepare ca. 500-1000 ppm stock solutions in an appropriate solvent such as acetonitrile. Dilute to proper working standard concentrations (ca. very low ppm levels) in a solvent compatible with the mobile phase.

Standards for GC-MSD Analyses. Prepare ca. 500-1000 ppm stock solutions in an appropriate solvent such as chloroform. Dilute to proper weight/volume working standard levels (to ca. very low ppm levels) in proper extraction solvent.

Standards for HS-GC-MSD. Mixed stock standards - transfer each solid or viscous liquid analyte in 50 - 100 mg portions into a tared headspace vial. Weigh vial after each addition, and fill vial with known volume of appropriate solvent, seal vial, reweigh the vial and contents and calculate the weight/weight concentration of each analyte. For volatile analytes, using a 100 µL syringe, add each analyte in 50 - 100 µL portions to a septum sealed headspace vial containing a known volume and weight of appropriate solvent such as methanol. Weigh vial after each addition, and calculate the weight/weight concentration of each analyte. Dilute to proper weight/volume working standard levels (down to ca. 0.2 ppm) in methanol.

Instrumentation

Ionizing Chambers and Radiation Sources. Test materials were e-beam or γ-irradiated at ambient temperature in sealed headspace vials. E-beam exposures were preformed at Steris Isomedix Services, Morton Grove, IL and Libertyville, IL. Test specimens were irradiated at a dose rate of approximately 5 kGy per second for 5 kGy target dose, and of 10 kGy per second for 25 and 50 kGy target doses. Dose absorbed by the test materials was determined using radiochromic films according to ASTM method E1275 (*5*). The specimen bottles were packaged in plastic sleeves, which were heat sealed in a pouch. The pouch was hung on a Sample Presentation Assembly (a solid board) to assure the samples would form a monolayer for the beam to penetrate. At each target dose, there was a single dosimeter placed on the surface of the pouch in the center. Some test specimens were γ-irradiated at 25 and 50 kGy with a Gammacell irradiator (a ^{60}Co-gamma source) at the National Institute of Standards and Technology (Gaithersburg, MD, USA) at a dose rate of ca 6 kGy/h, in ambient air.

HPLC-MSD-Data System (DS) for Analysis of Non-volatile and Semi-Volatile Analytes. Agilent 1100 HPLC-MSD with atmospheric pressure chemical ionization interface. Column - 150 mm x 2 mm Phenosphere C-8, 3 µm particle size, 80 Å pore diameter (Phenomenex, Torrence, CA). Mobile phase - methanol/water. LC conditions: injection volume 5 µL, column temperature 45°C, flow rate 0.5 ml/min, various gradient profiles with mobile phases A (0.1% formic acid in water/acetonitrile (95:5, v/v)) and B (0.1% formic acid in acetonitrile), prepared by diluting 1.26 g formic acid 96% to 1 liter. For the quantitative analyses the following gradient was used: 10% B (0 to 0.5 min), then linear to 100% B (0.5 to 10 min), then held at 100%B (10 to 16 min). MSD APCI (Interface) parameters: drying gas flow 5 L/min, nebulizer pressure 60 psig, drying gas temperature 350°C, vaporizer temperature 500°C, capillary voltage 2000 V, corona current 20 μA in negative (3 μA in positive) ionization mode. MS parameters: polarity: positive or negative (negative for the acids); fragmentor voltage 150 V; electron multiplier voltage 1400 V; gain 3.0, dwell time 229 msec. Scan parameters: scan range 100-450 (during the first 7 min), 100-900 (7 to 12 min).

HPLC-DAD-DS for Analysis of Non-volatile and Semi-volatile analytes in Migration Solutions. HPLC system consisting of a Waters 600E system controller interfaced with a 9100 Varian Autosampler, Waters 486 tunable Ultraviolet (UV) detector, Varian 330 Photo diode Array (PDA) detector, and Varian Star Workstation software. A Symmetry C_8 column (Waters, Milford, MA) with an acetonitrile/ water gradient mobile phase was used. The linear gradient starting at 90% water and going to 0% water in 15 minutes was typical. More specific details are described by Komolprasert, et al. (*6, 7*).

GC-MSD-DS for Analysis of Semi-volatiles in Extracts of Polymers. Hewlett-Packard 5890 GC-5970B MSD and Chemstation operating software. A 30 m x .25mm i.d. HP-5MS FSOT capillary (an ultra low bleed 5% diphenyl - 95% dimethyl siloxane liquid phase), $d_f = 0.25$ μm (#19091S-433, Agilent Technologies, Wilmington, DE). In 22 mL headspace vials, 1.0 g polymer is extracted in 10 mL of a non-dissolving penetrating solvent. The polymers are extracted for 24 hours at room temperature on an end-over-end rotary mixer. Aliquots are directly injected or concentrated and solvent exchanged before analysis. All calibrations are external. Some additional information on the procedures is described by McNeal, et al. (*8*).

HS-GC-MSD-DS for Analysis of Volatiles. For the analysis of a broad range of volatile chemicals, a Hewlett-Packard 5890 GC-5970B MSD and Chemstation operating software was used with a 30m x .25mm i.d., $d_f = 1.4$ μm ZB-624 capillary (#7HG-G005-27, Phenomenex, Torrance, CA). Cryofocusing was employed with splitless injections. For very light molecules such as acetaldehyde and gases, a Perkin-Elmer HS-40 automated headspace sampler (Perkin-Elmer Corp, Norwalk, CT) coupled to an Agilent Technologies 6890 capillary GC with an Agilent Technologies 5973 Performance Turbo MSD (Agilent Technologies, Wilmington, DE) was used. The capillary column employed with this system was a 15 m x .32 mm i.d. HP PLOT Q capillary, $d_f =$ ca. 20 μm (Agilent Technologies #19091P-Q03). HS-40 operating parameters – 1 hour equilibration time at 150 °C for olefin-phthalate based polyesters and 125 °C for all other polymers, needle and transfer line temperatures - 150 °C, high pressure vial pressurization - 0.5 min at 30 psi, injection time - 0.2 min. GC conditions - temperatures (°C) - injector - 200, interface - 180, constant column flow 1.7 mL/min (1.7 psi at 30 °C), split vent open after 0.75 min. Oven program - 1 min at 50 °C, 10 °/min to 200 °C, hold 12 min. Total run time = 30 min. MSD parameters - Autotune calibration, temperatures source - 230°C, quadrupole – 150°C, scan mode, range - 25 - 200 daltons, scan rate - 2.14 scans/sec.

Preparation of Test Specimens for Irradiation and Analysis. Polymer test materials were either fine powders or sheet stock cut into strips. For the analysis of volatiles, polymers were usually cryogenically ground using dry ice and an ultracentrifugal mill equipped with a 0.5 mm sieve. In 20 mL headspace vials, test specimens were vacuum dried overnight at 40-45°C, then flushed with purified air and sealed with PTFE faced septa. Ten replicates were prepared for each radiation exposure dose, and ten control polymer specimens (non-irradiated (NIR)) were prepared for each polymer test material. Then the test specimens in the sealed vials were irradiated at room temperature. After irradiation, the test specimen vials were kept in a refrigerator maintained at ca. 6 °C until analysis. Additional details are further described by Komolprasert, et al. (*6, 7*) and Buchalla, et al. (*9 - 11*).

Analysis of Polymer Specimens and Extraction Solutions. Solvent extraction of test specimens for semi- and non-volatiles was usually performed in the original headspace vial. As much as 20 mL of solvent can be added to a 1 g specimen in a vial. When a larger sampling is required the specimens from more than one vial were combined and extraction performed in a larger vessel such as a 100 mL round bottom flask. After extraction, solutions were concentrated over steam and analyzed by HPLC-MSD, HPLC-DAD or GC-MSD. Vials of test specimens were directly analyzed for volatiles by HS-GC-MSD. These procedures are described by Komolprasert, Begley, Buchalla and McNeal (*6-12*).

Data Analysis. Identity of chemicals was based on retention time, spectra of authentic standards and structural elucidation from mass spectral characteristics of chemicals of similar structure to the unknowns. Otherwise, identification was tentative. Quantitative analysis was based on authentic and deuterated standards or external standardization with chemicals of similar structure to unknowns.

Results and Discussion

Polymer Analyses. Sheets of amorphous polyethylene terephthalate (PET) bottle resin were γ–irradiated at 25 and 50 kGy. The specimens, including the non-irradiated controls, were extracted with acetone; the extracts were concentrated and analyzed for nonvolatile monomers and oligomers by HPLC-MSD. Also, a 1 g aliquot of the amorphous PET sheets was dissolved in 1 mL of a 30% solution of hexafluro-isopropanol in methylene chloride, the polymer reprecipitated with methanol, and the extract concentrated and analyzed by HPLC-MSD. Results of the analyses show both the extraction and dissolution procedures produced equivalent data. Also, for the most part, there were no differences between the chromatograms of the irradiated and non-irradiated specimens. The one difference is shown in Figure 1. In the Figure, overlaid HPLC total ion chromatograms (TIC's) from analyses of extracts of reprecipitated PET are shown. The chromatographic region depicted shows equivalent responses for the peaks of the non-irradiated polymer and the polymer after exposure to 25 kGy and 50 kGy of γ–radiation except for the peak at ca. 6.3 min. This peak was identified as the monoethyl ester of terephthalic acid (m.w. = 194). Quantitatively the data show the level of the monoethyl ester increased from 0.7 ppm in the non-irradiated polymer to 1.7 ppm in the polymer after exposure to 50 kGy of γ-radiation. Other than the increase in the level of the monoethyl ester as the level of γ–radiation increased, the data show very little change in chromatographic responses for all of the other peaks including the PET linear dimer at ca. 6.6 min for all the extract analyses. Also, chromatograms from the DAD in series before the MSD showed the same number of peaks as seen in the MSD chromatograms. This fact suggests that the LC-MSD ionization conditions were appropriate for measuring many non-volatile PET based chemical products formed as the result of exposure to ionizing radiation. The UV and MSD data show that very little if any non-volatile by-products are formed when food grade PET is exposed to up to 50 kGy of γ–radiation.

In another experiment, test specimens of food grade HDPE were γ-irradiated at the 25 kGy dose level, the specimens and controls were extracted with isopropanol following ASTM Method D-5524 (*13*), the extracts concentrated and semi- and non-volatile components determined using HPLC-MSD. Figures 2 and 3 show the resulting TIC's from the analyses. The Figures show the first and second half of the chromatogram from the analysis of the extract of the non-irradiated (NIR) polymer overlaid by the chromatogram from the analysis of the extract of the polymer after exposure to 25 kGy of γ–radiation. Figure 2 shows the late chromatography resolving the antioxidants. It is obvious that 25kGy exposure of γ–radiation reduces the amounts of available antioxidants in the HDPE. In fact after irradiation, Irgonox 1010 and Irgofos 168 are not even detectable. The early chromatography is shown in Figure 3. In the 8 to 9 min region of the chromatogram, we see what we believe

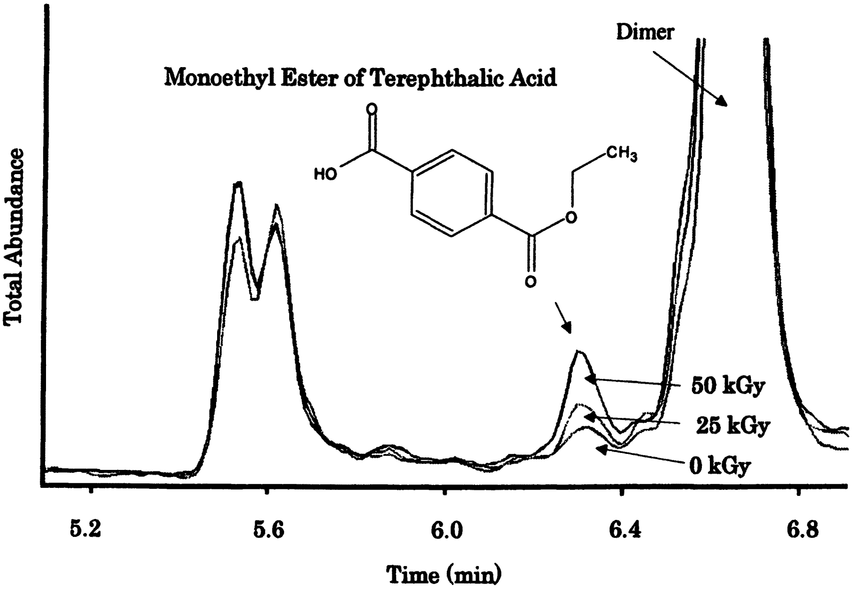

Figure 1. HPLC-MSD chromatograms of non-volatile extractables from PET before and after exposure to different doses of γ-radiation.

are the breakdown products of the antioxidants seen in Figure 2. As expected we see greater amounts of the breakdown products in the irradiated polymer extract and lesser amounts in the non-irradiated polymer extract. The proposed structures of the co-eluting oxidation products are shown in Figure 3. Di-t-butyl phenol was confirmed with a standard and most likely is an oxidation product of Irgafos 168. This finding is in agreement with results from other studies on radiation by-products of HDPE polymers presented at this Symposium by Deschenes, Kawamora and Franz along with the findings of other investigators (*11, 14-20*). Another rather dramatic conclusion drawn from this experiment is the rapid and efficient reduction in the levels of antioxidants and the equally rapid and efficient increase in the levels of by-products formed in the HDPE matrix after exposure to 25 kGy of γ-radiation. When antioxidants were regulated for food contact use it was not perceived that they would totally decompose forming a whole new group of chemicals in a very short period of time.

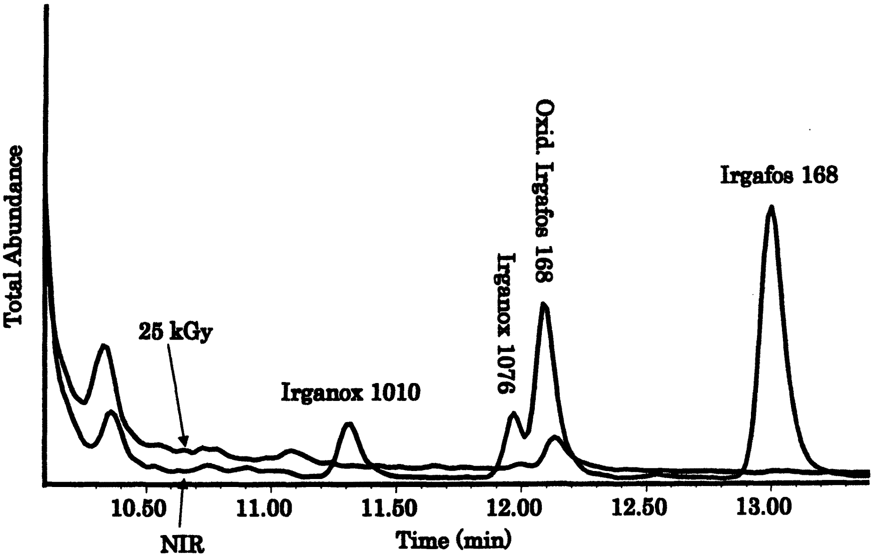

Figure 2. Late eluting portion of HPLC-MSD chromatograms of HDPE extract before and after exposure to γ-radiation.

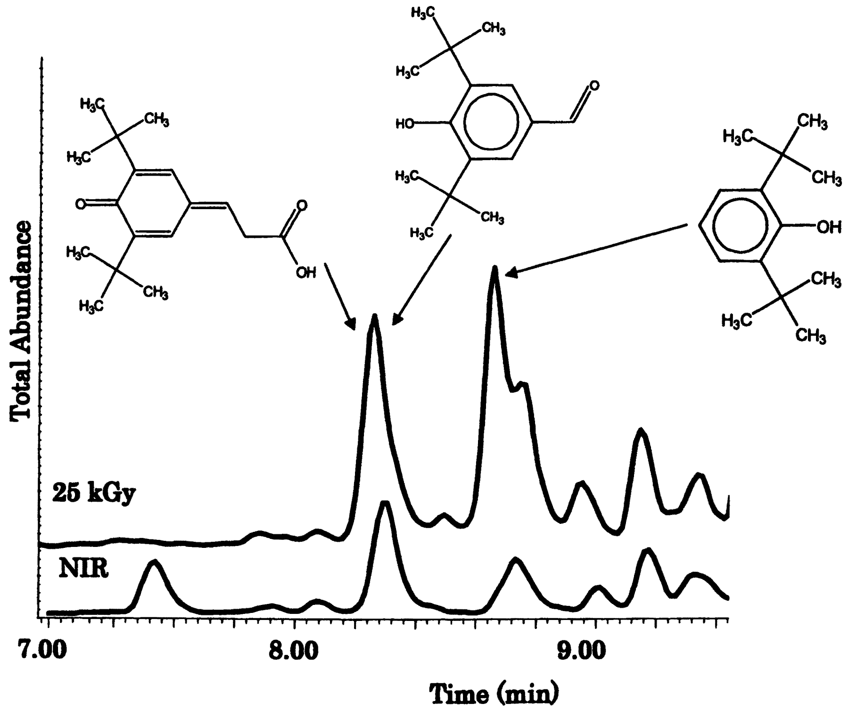

Figure 3. Early eluting portion of HPLC-MSD chromatograms of HDPE extract before and after exposure to γ-radiation.

Migration Experiment. To evaluate and compare the effects of γ- and e-beam radiation on polymers, two sets of ethylene-terephthalate co-polyesters (PETG), one containing three and the other containing thirty one mole percent cyclohexane-1, 4-dimethanol (CHDM) co-monomer were exposed to 5, 25 and 50 kGy of γ-radiation and two more identical sets were exposed to 5, 25 and 50 kGy of e-beam radiation.

These two polyesters were amorphous CHDM-ethylene-terephthalate co-polyesters with the following properties: The first co-polyester was comprised of 3.0 mol % CHDM co-monomer and 1.5 mole % diethylene glycol, and 95.5 mole % ethylene glycol. The finished polymer had a 1.37 g/cc density, 5% crystallinity, intrinsic viscosity (IV) 0.8 and 0.25-0.28 mm thick. The second co-polyester was 31 mole % CHDM and 69 mole % ethylene glycol. The polymer had a 1.33 g/cc density, less than 5% crystallinity, IV 0.73 and 0.25-0.28 mm in thickness. The composition of the CHDM comonomer in these co-polyesters was supplied by, Eastman Chemical Company (Kingsport, TN), the manufacturer of the co-polyester, and was not confirmed analytically. Both materials were in a roll stock of approximately 61 cm in width.

Following irradiation these materials were extracted for nonvolatile monomers and oligomers by dissolving and precipitating the polymers using hexafluroisopropanol then acetone as outlined by Begley, et al. (*12, 21*), and the extracts were analyzed using HPLC with DAD. In another experiment, the remaining test specimens exposed to e-beam radiation and controls were extracted for ten days at 40 °C using n-heptane and 10% ethanol in water, food simulating solvents. Then migrating monomers and oligomers were determined by HPLC with DAD.

Results of the analysis of the extracts from the different co-polyesters for UV absorbing residual nonvolatile monomers and oligomers to compare the effects of γ–radiation and e-beam radiation show the two ionizing radiation techniques generated the same chemicals in equivalent amounts. More importantly, the same quantities of nonvolatile monomers and oligomers were extracted from the controls as were extracted from the co-polyesters after exposure to ionizing radiation at any dose level, which indicates no measurable effects by either radiation technique.

In the next experiment, the levels of nonvolatile monomers and oligomers migrating into two different food simulating solvents from the two PETG co-polyesters after exposure to e-beam radiation were compared. Only the cyclic trimer and mono-hydroxyethyl terephthalic acid migrated in detectable quantities. Table I summarizes those migration results and the corresponding e-beam data on extractable residuals. The cyclic trimer residuals were ca. 20% higher in the co-polyester made with 3% CHDM than measured in the other co-polyester. Also, levels of mono-hydroxyethyl terephthalic acid migrating into n-heptane were not detectable, whereas, detectable levels were measured into 10% ethanol. The difference in migration between n-heptane and 10% ethanol is most probably related to solubility effects. One other difference observed was migration of the cyclic trimer. Averaged levels migrating from the co-polyester made with 3% CHDM into n-heptane were four times more than into 10% ethanol, and for the co-polyester made with 31% CHDM, migration into n-heptane was two times more than into 10% ethanol. Also, the data show that even though the levels of non-volatiles extracted from the two co-polyesters ranged from the low ppm up to the low parts-per-thousand only very low ppb levels migrated into food simulating solvents.

Table I. Low Molecular Weight Chemicals from PETG Co-Polyesters after Exposure to e-Beam Radiation

Analyte	*Residuals (ppb)*	*Migrating* [1] *(ppb)*
Terephthalic acid	ca. 1,000	<1
Mono-hydroxyethyl terephthalic acid	ca. 5,000	ca. 1
Bis-hydroxyethyl terephthalate	ca. 20,000	<1
Cyclic trimer	ca. 5,000,000 [2]	ca. 2 [3]

[1] Average levels for both 10% EtOH and n-heptane.

[2] Levels in 3% CHDM polymer ca. 20% higher than 31% CHDM.

[3] Averaged levels into n-heptane were 3 to 4 fold greater than into 10% ethanol.

Analyses for Semi-volatiles. Limited studies have been conducted on extractable semi-volatiles in irradiated polymers, but no data are presented here. Analyses were conducted for semi-volatiles in polymers after exposure to γ-radiation by Buchalla (*11, 14, 15*). The results quickly show that the data from semi-volatiles analyses complemented the data from both the volatiles and the non-volatiles analyses. Many of the chemicals too volatile for LC-MSD detection and not volatile enough for HS-GS-MSD were detected by GS-MSD as extractable semi-volatiles. Polymers are routinely extracted and analyzed for semi-volatiles using capillary GC with mass selective detection. The analysis is part of the identification process of food packaging components, and we plan to further investigate the effects of ionizing radiation on food contact materials and characterize the semi-volatile chemicals present. Also, when one considers the advent of HPLC-MS in the laboratory and its complexity, GC-MSD with electron impact ionization, can be used to assist in the identification of late eluting semi-volatiles, which can be difficult to identify with HPLC-MSD.

Analysis for Volatiles. Volatiles present in the test specimens were sampled using a static headspace technique performed under phase equilibrium conditions (solid-vapor) predetermined for the volatiles present in the test specimens. The static headspace methods used are based on ASTM Methods D-4526 (*22*) and F-2013 (*23*). In method development work with ASTM Method 2013-01, it was found that static headspace sampling after thermal equilibration at 150 °C for 1 h was the best compromise of time and temperature that minimized thermal degradation and further formation of acetaldehyde from terephthalate polyesters. For manual headspace sampling, the maximum safe equilibration temperature with minimum condensation within the syringe was 125 °C. Although 1 h at 150 °C were found to be the optimum time/temperature conditions for solid-vapor phase partitioning of actetaldehyde, 1 h at 125 °C was adequate for analyte identification. Volatiles present in irradiated polymers were determined using manual static headspace sampling, capillary GC with a narrow bore, wall coated open tubular, capillary and mass selective detection. Additionally, a Perkin-Elmer automated headspace sampler was coupled to an Agilent 6890 capillary GC, which was further coupled to a 5973 high performance turbo MSD. Because of the high performance turbo, a short wide bore porous layer open tubular (PLOT) capillary was able to be used operating at an optimum helium carrier gas flow rate of 1.7 mL/min in the splitless injection mode. Thus, gases such as vinyl chloride (t_r ca. 5 min) was able to be retained without cryofocusing.

Figure 4 shows overlaid PLOT chromatograms of volatiles from the headspace analysis of the PETG co-polyester made with 3% CHDM before (lower chromatogram) and after exposure to e-beam radiation at the 50 kGy dose level. Both chromatograms show sharp distinct peaks, and stable baselines. For the most part, the peaks for the volatile chemicals in both non-irradiated and irradiated polymers are identical, and only differ in magnitude. Noticeable differences seen in the chromatograms are the acetaldehyde, ethyl acetate and formic and acetic acid levels increase after irradiation, whereas 2-methyl-1, 3-dioxolane levels decrease after exposure to a 50-kGy dose. The quantitative analysis for a pair of volatile components in the co-polyester is shown in Table II. As the table shows, the levels of acetaldehyde steadily increase up to 10.7 ppm after the PETG is exposed to 50 kGy of e-beam radiation, whereas, the levels of 2-methyl-1, 3-dioxolane steadily decrease to a low of 1.1 ppm after the PETE is exposed to 50 kGy of e-beam radiation.

Table II. Concentration (ppm) of Acetaldehyde and 2-Methyl-1, 3-Dioxolane in PETG Co-Polyester

e-beam dose level	*Acetaldehyde*	*2-Methyl-1, 3-Dioxolane*
non-irradiated	1.8	8.2
5 kGy	3.4	5.1
25 kGy	6.1	2.5
50 kGy	10.7	1.1

In another experiment, the two different PETG co-polyesters exposed to different dose levels of either γ- or e-beam radiation were analyzed for volatiles using HS-GC-MSD. It was felt that any differences in the effects of exposure to the two radiation sources would be reflected in changes in the number and

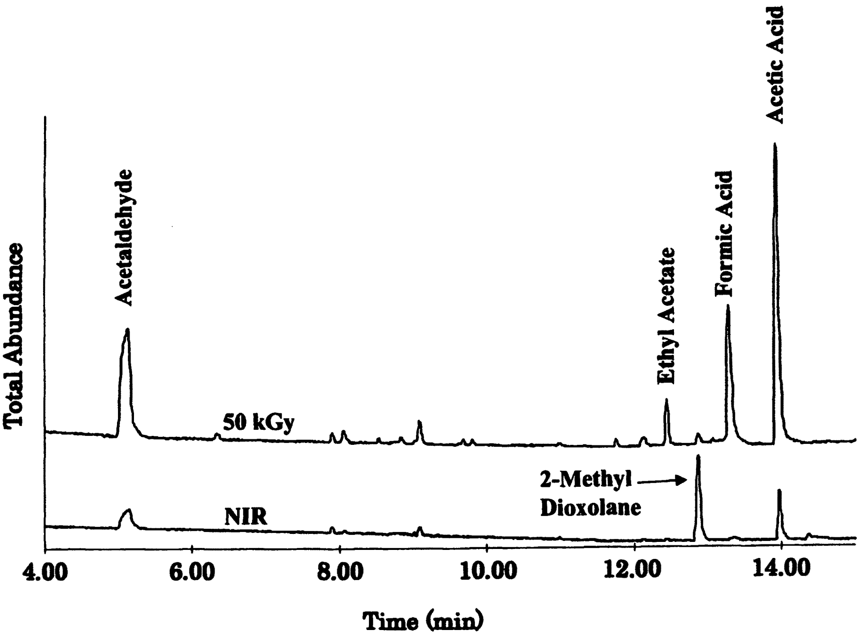

Figure 4. GC-MSD PLOT chromatograms of headspace over PETG co-polyester before and after exposure to e-beam radiation.

amounts of volatile chemicals formed. The resulting chromatograms showed that the number of volatiles formed after the two co-polyesters were exposed to the different radiation sources were the same for equivalent dose levels. Also for the most part, the relative intensities of the chromatographic peaks were essentially the same for equivalent dose levels. No differences were seen in the volatiles profiles of the two co-polyesters after exposure to e-beam and γ-radiation.

In Figure 5, overlaid headspace chromatograms are shown from the analysis of an EVOH copolymer using the PLOT column. The lower chromatogram is from the analysis of the control polymer and the upper chromatogram represents the headspace analysis of the copolymer after exposure to a 50-kGy dose of e-beam radiation. The number of peaks in the control chromatogram is easily determined, and the number of volatiles in the polymer is small. When the copolymer is irradiated the volatiles created are too numerous to count. The chromatograms show that when the EVOH is irradiated it breaks down, producing many volatile chemicals from hydrocarbons to acids. It can be seen that the levels of many of the chemicals identified in the EVOH copolymer increase dramatically after exposure to ionizing radiation, but it is difficult to say that any of the chemicals identified are uniquely by-products from the exposure of the EVOH to ionizing radiation. Many of the chemicals identified in the irradiated polymer potentially could be present in the non-irradiated copolymer but their levels were below the detection limit.

The next polymer investigated was polyamide 6I/6T (poly- (hexamethylene isophthalamide/hexamethylene terephthalamide)). Polyamide 6I/6T is a condensation polymer formed when a combination of *meta*- and *para*-phthalic acids are reacted with the co-monomer, hexamethylene diamine. Figure 6 displays overlaid chromatograms from the analysis of volatiles formed in the polyamide before and after exposure to different doses of e-beam radiation. The chromatographic analyses were performed with the ZB-624 capillary. The column is slightly polar for the retention of polar analytes and allows for the elution of many semi-volatiles within a reasonable time. The chromatograms are similar to the chromatograms of volatiles from the analysis of the PETG co-polyester in that there are very few volatiles formed and their intensities are quite low. Judging from the number of chromatographic peaks and their magnitudes, even after the polymer is exposed to a 50-kGy dose of e-beam radiation, this polyamide remains quite stable. Very few volatile chemicals are formed when the polyamide is exposed to e-beam radiation. Table III summarizes the quantitative analysis for volatile chemicals in the polyamide 6I/6T. The data show little if any residues in the non-irradiated specimen, but when the polymer is irradiated the amounts of chemicals increase as the dose level increases.

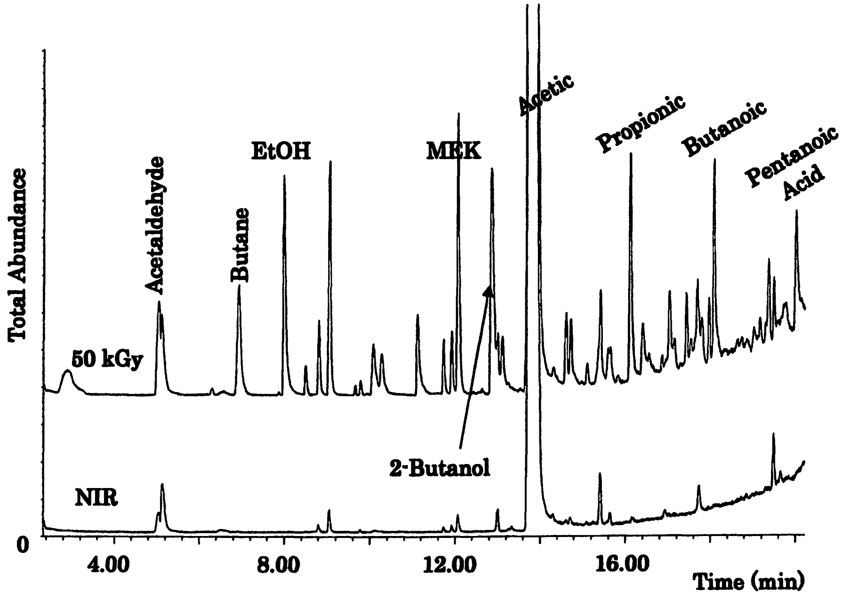

Figure 5. GC-MSD PLOT chromatograms of headspace over EVOH co-polymer before and after exposure to e-beam radiation.

Table III. Quantitative Results (ppm) for Volatile Chemicals in Polyamide 6I/6T before and after Exposure to e-Beam Radiation

Analyte	*e-Beam Dose Level*			
	Non-irradiated	*5 kGy*	*25 kGy*	*50kGy*
n-Butanal	n.d[1]	0.3	0.8	1.8
Methyl ethyl ketone	n.d.	n.d.	0.9	1.7
Acetic acid	n.d.	n.d.	12.1	29.5
n-Pentanal	1.0	7.1	29.9	72.1
Methyl cyclo-pentene-1-one[2]	n.d.	n.d.	0.5	2.2

[1] not detected.

[2] Identity based on best spectral library fit.

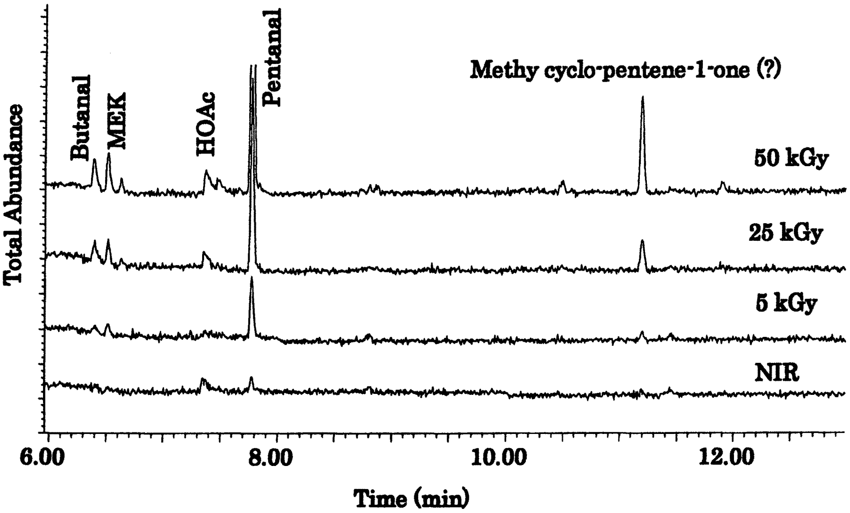

Figure 6. GC-MSD chromatograms with the ZB-624 FSOT column of headspace over polyamide 6I/6T before and after exposure to e-beam radiation.

A sample of polyamide 6 was also analyzed for volatile chemicals before and after exposure to γ-radiation. These analyses were also performed using the ZB-624 capillary column. Like the polyamide 6I/6T analyses the chromatographic peaks were sharp and well resolved, and the intensities of the peaks increased as the radiation dose increased. There were more chromatographic peaks in the irradiated polyamide 6 than were seen in the irradiated polyamide 6I/6T. The monomer, caprolactam, was the only well defined peak in the chromatogram of the non-irradiated polymer. It is a semi-volatile and enough partitioned into the vapor phase to be easily detected in the volatiles chromatogram. The level of caprolactam in the polymer is in the high ppm range. After exposure to 50 kGy, the level of caprolactam tripled. After exposure to γ-radiation, the polymer "unzips" forming more of the monomer. The other major volatile components seen in the irradiated specimens and not detected in the controls were n-butanal, n-pentanal and pentanamide, which are most likely breakdown products of the monomer, caprolactam.

Summary

Data on potential migrants from a limited number of irradiated polymers have been presented. Also, results of migration experiments from two ethylene-terephthalate co-polyesters have been presented. In the presence of oxygen, polymers were irradiated with γ- or e-beam radiation, extracted with select solvents and food simulating solvents and the extracts analyzed by HPLC-MSD or HPLC-DAD. In the case of PET, the level of one non-volatile residual monomer more than doubled (from ca. 0.7 to ca. 1.7 ppm) after exposure to 50 kGy of γ-radiation. Other than the increased level of the non-volatile monomer, little difference was seen in the number and amounts of residual non-volatile chemicals extracted from the PET specimens before and after irradiation.

Two PETG co-polyesters were exposed to either γ- or e-beam radiation, extracted with solvent and different food simulating solvents, and the extracts were analyzed for non-volatile monomers and oligomers by HPLC-DAD. The chromatograms of these extracts show no differences in the number and amounts of residual non-volatile chemicals extracted from the different PETG co-polyester specimens before and after irradiation. There were no differences in the number and levels of UV absorbing non-volatiles extracted after exposure to equivalent doses of e-beam and γ-radiation. Also, analysis of solutions from migration tests showed no increases in the number and amounts of non-volatile monomers and oligomers as the result of exposure to e-beam radiation up to 50 kGy. In summary, the levels of residual monomers and oligomers approached the low parts-per-thousand levels, whereas very low parts-per-billion quantities migrated.

HDPE specimens were exposed to a 25-kGy dose of γ-radiation, the specimens were extracted with solvent and the extracts analyzed for antioxidants (polymer additives) using HPLC-MSD. Results of the HPLC-MSD analyses show complete elimination of Irganox 1010 and Irgafos 168, and a noticeable reduction in the amount of Irganox 1076 in the HDPE after exposure to 25 kGy of γ-radiation. One of the by-products, the hindered phenol, could be a breakdown product of either of the Irganox's or of the Irgafos's. Other chemicals, whose mass spectra suggest a hydroquinone-allyl-acid and a hindered phenol-aldehyde were tentatively identified in the extract of the irradiated HDPE and are suspected to be oxidation products of the antioxidants. Currently, these antioxidants are approved for use in food contact polymers. They are not approved for use in food contact polymers exposed to ionizing radiation. It was not envisioned that most or all of an antioxidant would decompose in the typical life cycle of a food contact article as was seen in the irradiated HDPE. This phenomenon requires a closer look into the identification, the amounts and the dietary exposure to those by-products formed from antioxidants in polymers used to prepackage foods prior to irradiation. It is not known if the same phenomenon occurs with other polymer additives, and experiments should be performed to determine the stability of other polymer additives when exposed to ionizing radiation.

Ionizing radiation increases the number and amounts of volatiles in polymers. Results of analyses for volatiles in the two PETG co-polyesters show no differences in the number and relative amounts of volatiles formed after exposure to equivalent doses of e-beam and γ-radiation. In the case of polymers with an aromatic component, the number and amounts of volatiles produced as the result of exposure to ionizing radiation are much lower than seen in an aliphatic based polymer. Specifically PET, PETG and polyamide 6I/6T are much more stable when exposed to ionizing radiation than polyamide 6 and much more so than an EVOH copolymer. Most of the volatiles seen in irradiated polymers are reduction or oxidation products of the alkyl components of the polymers. With the possible exception of the EVOH copolymer, the amounts of volatiles produced during the irradiation of the polymeric materials evaluated in this study are insignificant, i.e., very low ppb levels would be expected to migrate into foods. Most of the chemicals found are thought to also be products of the polymerization process or conversion of the polymer(s) into a finished package, and breakdown products of polymer additives. Although there is an increase in the concentration of some of these chemicals when the polymers are exposed to ionizing radiation, few of the chemicals are thought to be specifically and uniquely formed by irradiation.

Work remains to be done on the identification and amounts of by-products in food contact polymers and additives after exposure to ionizing radiation. Currently the irradiation of polymer additives such as hindered phenol antioxidants, colorants and nitrogen containing UV stabilizers and their by-products are being investigated. Other polymer-additive systems that are of interest include plasticized PVC. Both the homopolymer, the plasticizers and the by-products formed after exposure to ionizing radiation are of interest. Also, plasticizers are used in acrylics, styrenics and vinylidene chloride co-polymers, the latter two of which are approved for irradiation, and little work has been done showing the effects of ionizing radiation on those plasticizers. Another class of polymers (and additives) of potential interest for future investigations is adhesive formulations. Currently none are approved for the prepackaging of food prior to exposure to ionizing radiation.

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October 23, 2009 | http://pubs.acs.org
Publication Date: January 2, 2004 | doi: 10.1021/bk-2004-0875.ch015

Chapter 15

Effect of Ionizing Radiation on the Migration Behavior and Sensory Properties of Plastic Packaging Materials

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Irradiation of packaging materials - in most cases plastics - generally leads to a formation of free radicals and ions, with secondary effects such as cross-linking as well as oxidative chain scission. As a consequence, radiolysis products are generated which may induce off-odors in the packaging polymers as well as changes in migration characteristics. E.g. during irradiation, polymer additives may be destroyed, which may change the specific migration of additives and additive related decomposition products. The effects may have consequences on the quality and safety of the packed goods. The aim of this work was the investigation into irradiation induced migration in various packaging materials and a (semi)-quantification of radiolysis products. In addition, the impact of ionizing radiation on sensory properties of the packaging materials was determined. The results are discussed in view of food packaging legislation requirements.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Introduction

Sterilization of packaging materials with ionizing radiation is a principal alternative to other sterilization methods e.g. chemicals or heat treatment. For this purpose the packaging materials are pre-sterilized in commercial irradiation plants with ionizing radiation from ^{60}Co sources or electron beams from electron accelerators, respectively. Subsequently, the pre-sterilized packaging materials are filled in aseptic filling lines with foodstuffs. For several packaging materials, e.g. bag in box systems, irradiation is the only suitable sterilization method. However, besides specific regulations in some EU member states for irradiated goods, a low consumer acceptance of irradiation in general as well as possible effects of ionizing radiation on packaging polymers and additives lead to a currently low market penetration of irradiated packaging materials. From our point of view, rigorous specific regulations from authorities for radiation sterilized packaging materials are correlating with the lack of information on migration data of irradiated packaging materials. The key question, which should be answered, is: does irradiation cause a migration potential which can conflict with legal requirements for food packaging materials?

In general, irradiation of polymers may lead to a formation of free radicals and ions, with secondary effects such as cross-linking as well as oxidative chain scission. These effects usually result in formation of volatile radiolysis products which may induce off-odors in the polymers. In addition, the migration characteristics of packaging materials principally might be changed with possible consequences on the quality and safety of packaged goods. With increasing absorbed irradiation dose also polymer additives may be destroyed which may affect the specific migration behavior of additives and their related decomposition products. In the last three decades several publications have been dealing with the impact of irradiation on polymers and the formation of radiolysis products (see for example *1, 2, 3, 4, 5, 6, 7, 8, 9, 10* and literature cited therein).

The aim of our investigations was a (semi-) quantification of radiolysis products in sterilized packaging materials as well as changes in concentration of polymer additives or polymer constituents (e.g. polyolefin oligomers). On the basis of these results the interaction between packaging materials and food (simulants) were evaluated. Our previous work *(11, 12, 13)* investigated several monolayer packaging films e.g. polyethylene (LDPE), polypropylene (PP), polyamide (PA), polystyrene (PS), polyvinylchloride (PVC) and polyethylene terephthalate (PET). The current work investigates the impact of ionizing radiation on containers for soft-drinks, dairy products or pharmaceutical packaging made of LDPE, PP, PS and PET. For all packaging materials investigated in this study, sterilization with ionizing radiation may be a future option instead of conventional sterilization with chemicals.

Experimental

Packaging Materials

The packaging materials investigated in this study were supplied by packaging manufacturers. Four different polymers were chosen for this study. Polyethylene terephthalate (PET) bottles which are typically used for soft-drinks, juices or mineral water. Polypropylene (PP) and polystyrene (PS) cups, both are used for yogurt or other dairy products. Finally, low density polyethylene (LDPE) was chosen, which is widely used as packaging material for foodstuff or pharmaceutical products. All packaging materials investigated in this study were pre-sterilized by ionizing radiation. All samples were investigated in comparison to their unirradiated reference samples.

Irradiation of Packaging Samples

The samples were irradiated in commercial irradiation plants with ^{60}Co or electron beam. Table I gives an overview over the investigated samples and the applied radiation doses. Except for LDPE the packaging polymers were irradiated with high radiation doses in order to generate a sufficient worse-case scenario and to induce measurable effects. In the food packaging area, a typical dose for sterilization of the packaging material is approximately 10 kGy. In order to investigate the influence of the radiation dose, PP and PS were irradiated with two doses: 8.5 kGy which is applied in practice for sterilization and a worse-case dose of 23.9 kGy. LDPE was irradiated with the dose (15 kGy) used in practice for sterilization.

Table I. Investigated Polymer Samples and Applied Radiation Doses

Polymer	*Use*	*Applied radiation*	*Dose[kGy]*
PET	soft-drink bottles	^{60}Co	32.9
PS	dairy products	e-beam	8.5 and 23.9
PP	dairy products	e-beam	8.5 and 23.9
LDPE	food or pharmaceutical packaging	^{60}Co	15

Headspace GC/FID and GC/MS

Headspace gas chromatography (HS/GC) with either flame ionization detector (FID) or mass spectrometry detector (MS) was used for the screening of volatile substances and for quantification of radiolysis products in the polymers. Each sample was analyzed in the following way. The polymer sample (1.5 g for PS and PP, 1.0 g for LDPE and 0.7 g for PET) was cut into small pieces and placed in a 22 ml headspace vial. After equilibration for 1 h at appropriate temperatures (see below) the samples were analyzed by HS/GC with FID detection. Quantification was achieved by external calibration. Gas chromatograph: Perkin Elmer AutoSystem XL, column: J&W Scientific DB 1 - 30 m - 0.25 mm i.d. - 0.25 µm film thickness, temperature program: 50 °C (4 min), rate 20 °C min^{-1}, 320 °C (15 min), pressure: 50 kPa helium, split: 10 ml min^{-1}. Headspace autosampler: Perkin Elmer HS 40 XL, oven temperature: 120 °C (PP, LDPE), 150 °C (PS), 200 °C (PET), needle temperature: 140 °C (PP, LDPE), 170 °C (PS), 210 °C (PET), transfer line: 140 °C (PP, LDPE), 170 °C (PS), 210 °C (PET), equilibration time: 1 h, pressurization time: 3 min, inject time: 0.02 min, withdrawal time: 1 min.

Identification of volatile radiolysis products was achieved by HS/GC and MS detection. Gas chromatograph: Hewlett Packard 6890, column: Macherey-Nagel Optima 1 MS - 30 m - 0.25 mm i.d. - 0.25 µm film thickness, temperature program: 40 °C (5 min), rate 10 °C min^{-1}, 320 °C (1 min), pressure: 1.3 bar helium, split: 1:20. Mass spectrometry detector: Hewlett Packard 5973 Mass Selective Detector (MSD), MS-conditions: electronic ionization, full scan, scan range 34-700 daltons. Headspace autosampler: Perkin Elmer HS 40 XL, oven temperature: 120 °C (PP, LDPE), 150 °C (PS), 200 °C (PET), needle temperature: 140 °C (PP, LDPE), 170 °C (PS), 210 °C (PET), transfer line: 140 °C (PP, LDPE), 170 °C (PS), 210 °C (PET), equilibration time: 1 h, pressurization time: 3 min, inject time: 0.06 min, withdrawal time: 1 min. The mass spectra were compared for identification with the commercial NIST database.

Solvent Extraction and Gas Chromatography (GC/FID)

Semi-volatile radiolysis products were determined by gas chromatography with FID or MSD after extraction of the polymers. Extraction of PET: 1.0 g PET of each sample was transferred into glass vials. Then 2 ml of hexafluoro-*iso*-propanol (HFIP) as swelling agent as well as 10 ppm of methyl stearate (internal standard) were added. The vials were sealed and kept for 24 h at 60 °C with occasional agitation. The swollen PET was cooled to room temperature. After addition of 2 ml of *iso*-propanol as extracting solvent the vials were sealed again and kept for 24 h at 60 °C. Subsequently, the HFIP/*iso*-propanol extracts were decanted from the polymer. After 24 h at 4 °C the extracts were filtered using 0.2 µm pore size regenerated cellulose disposable filters and analyzed by GC/FID. Extraction of PS and PP: 1.5 g of the polymer samples were placed in glass vials and extracted for 24 h with 4 ml *n*-hexane at 60 °C. The extracts were filtered using 0.2 µm pore size regenerated cellulose disposable filters and analyzed by GC/FID. Extraction of LDPE: 1.0 g of the polymer samples was placed in glass vials and extracted for 24 h with 7 ml dichloromethane. The extracts were decanted and directly analyzed by gas chromatography without further sample preparation. Gas chromatograph: Hewlett-Packard HP 5890II, column: Supelco SE 10 - 30 m - 0.32 mm i.d. - 0.32 µm film thickness, temperature program: 40 °C (5 min), rate 15 °C min^{-1}, 240 °C (15 min), pressure: 50 kPa hydrogen, split: 10 ml min^{-1}.

For identification the extracts were analyzed by gas chromatography coupled with a mass detector. Shimadzu GC-17A gas chromatograph - QP-5000 mass spectrometer: Column: J&W Scientific DB 1 - 30 m - 0.25 mm i.d. - 0.32 µm film thickness, temperature program: 50 °C (2 min), rate 5 °C min^{-1}, 340 °C (10 min), injector temperature: 250 °C, interface temperature: 270 °C, injection mode: splitless. The mass spectrometer was operated under the following conditions: electronic ionization, scan range 40-700 daltons, scan rate: 0.60 s per scan. All data were recorded on a Shimadzu Class 5000 data system.

Sensory Examinations

The sensory examination was conducted by a trained panel of six testers. The applied method was in accordance to DIN 10955. Odor evaluation: the irradiated polymer samples were placed in a preserving jar and stored for 1 d at room temperature (23 °C). Subsequently, sensory differences were determined by the sensory panel. Taste evaluation: the irradiated test samples (yogurt cups) were filled with water and stored for 1 d at room temperature (23 °C). Subsequently, the differences between irradiated samples and the unirradiated reference samples were determined by the sensory panel. The applied evaluation scale ranges from I = 0 (no noticeable difference in odor/taste) to I = 4 (strong difference in odor/taste).

Results and Discussion

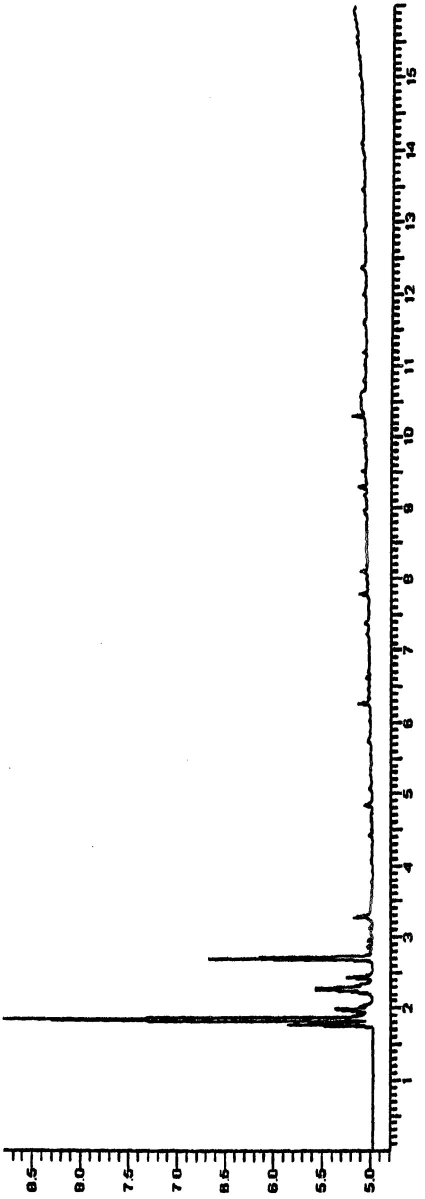
Volatile substances in the irradiated polymers were determined in comparison to the unirradiated reference samples by headspace gas chromatography (HS/GC). The applied HS/GC method detects preferentially volatile substances with molecular weight below 200 g mol^{-1}. Due to the higher diffusion of molecules with low molecular weight, these substances have a higher migration potential than the substances with higher molecular weights. The impact of irradiation on the formation of semi- or non-volatile substances e.g. polymer additives or lubricants was investigated after extraction of the polymers with suitable solvents. Both HS/GC and solvent extraction methods were developed such that their chromatograms have overlapping peaks; therefore no substance peak can be missed from detection.

Polyethylene terephthalate (PET)

Figure 1 shows a representative headspace gas chromatogram of PET soft-drink bottles irradiated at 32.9 kGy (^{60}Co) versus an unirradiated reference sample. In comparison, both irradiated and unirradiated gas chromatograms show only slight differences. Both gas chromatograms show PET typical substances including acetaldehyde (retention time R_t = 1.8 min), 1,3-dioxolane (R_t = 2.4 min) and 2-methyl-1,3-dioxolane (R_t = 2.7 min). These substances are generated in the melt phase during pellet and bottle production due to the high temperatures applied in the process. They were identified by GC/MS, and by comparing their retention times with those of the reference standards. Other substances in the irradiated PET samples could not be clearly identified by GC/MS because of their low concentrations and the unspecific mass fragmentation of such low molecular weight compounds. However, it was observed that irradiating PET bottles with 32.9 kGy changed concentration profiles of the detected substances. Acetaldehyde, for example, showed an increase up to approximately twice of the amount before irradiation. On the other hand, 2-methyl-1,3-dioxolane decreased significantly. Quantitative results for the investigated PET bottles are summarized in Table II. Due to only slight effects of irradiation on PET bottles, tests with more realistic (lower) radiation doses were omitted. It should be noted that all results are in agreement with a literature study (*9*).

In order to investigate the effect of oxygen during irradiation the same PET bottles were irradiated under nitrogen as an inert atmosphere. As a result, the modified atmosphere causes only slight effects on the concentration of the PET typical substances. For example, the increase of the acetaldehyde concentration is slightly lower than under irradiation at normal atmospheric conditions in the presence of oxygen. On the other hand corresponding to that, the decrease of 2-methyl-1,3-dioxolane is lower in the presence of oxygen.

In summary, irradiation of PET-bottles does not lead to a significant change in migration of relevant substances. The detected concentrations of volatile compounds are in the range typical for PET materials, also for virgin PET. New radiolysis products generated during the irradiation could not be detected in significant amounts. However, the concentration pattern of some typical PET contaminants is changed by the irradiation treatment.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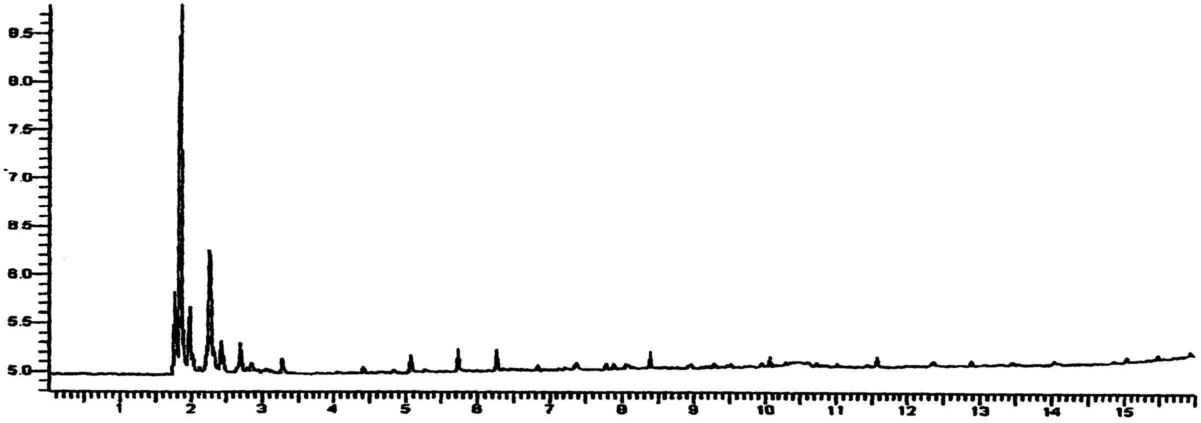

Retention time [min]

Figure 1. Representative headspace gas chromatograms of PET bottle (top: unirradiated reference sample, bottom: irradiated with 32.9 kGy)

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Table II. Results of the Quantification of Volatile Compounds in the Bottle Grade PET Material

Substance (R_t [min])	*Concentration ± standard deviation[a] [mg kg^{-1}]*		
	Unirradiated PET	*Irradiated PET 32.9 kGy normal atmosphere*	*Irradiated PET 32.9 kGy modified atmosphere (N_2)*
formaldehyde ? (1.77)[b]	3.0 ± 0.4	4.1 ± 0.3	4.4 ± 1.0
acetaldehyde (1.84)	13.4 ± 0.3	22.6 ± 3.6	19.7 ± 2.9
unknown (1.98)[b]	1.0 ± 0.1	1.8 ± 0.2	1.8 ± 0.4
unknown (2.27)[b]	1.9 ± 0.1	5.4 ± 0.6	3.6 ± 0.6
1,3-dioxolane (2.42)[c]	0.6 ± 0.1	1.5 ± 0.2	1.0 ± 0.2
2-methyl-1,3-dioxolane (2.69)	3.3 ± 0.1	1.2 ± 0.3	1.8 ± 0.4
unknown (3.28)[c]	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.2
unknown (4.84)[c]	0.2 ± 0.1	0.7 ± 0.1	0.3 ± 0.1

[a]standard deviation from three PET samples, [b]semi-quantification in comparison to acetaldehyde as standard. [c]semi-quantification in comparison to 2-methyl-1,3-dioxolane as standard.

Polystyrene (PS)

The headspace screening of the irradiated polystyrene cups (e-beam) in comparison to the unirradiated reference material shows qualitatively the same and quantitatively a similar fingerprint (Figure 2). Only some of the detected compounds are slightly different in concentration after irradiation. For example, the concentration of styrene (R_t = 6.4 min) decreases with increased irradiation dose whereas the concentrations of ethylbenzene (R_t = 6.0 min) and the oxidation product of styrene, acetophenone (R_t = 8.7 min), increase. Also the concentrations of unidentified, highly volatile substances with retention times between 1.7 min and 3 min increase with increased dose. Identification of these substances failed due to the unspecific mass fragmentation of the small molecules. In comparison to standard substances, however, the maximum molecular weight of these substances was estimated to be 100 g mol^{-1}. As shown in Figure 3 all substances could also be detected in the unirradiated reference samples. In contrast to the headspace screening, the gas chromatographic screening of dichloromethane extracts of irradiated versus unirradiated polystyrene samples shows insignificant changes in concentrations of the detected substances, predominantly polystyrene related oligomers, after irradiation. Semi-quantitative results as compared to methyl stearate (internal standard) are given in Table III.

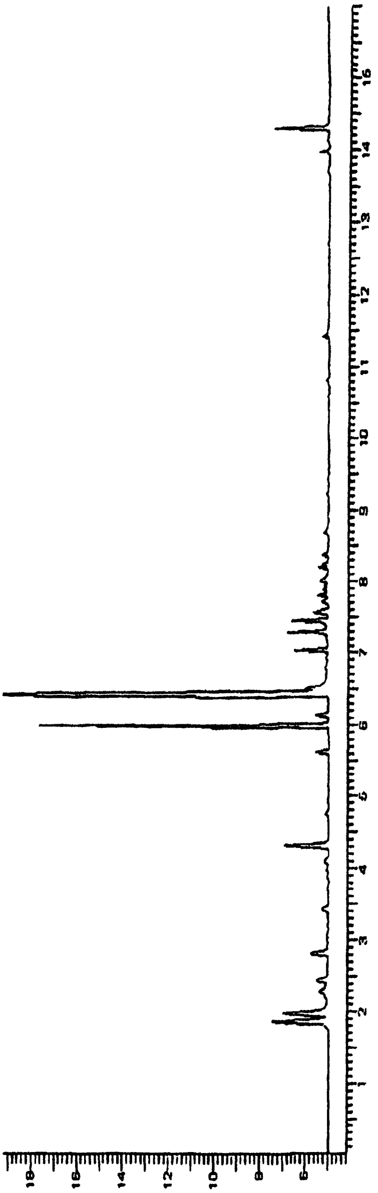
In summary, the investigated polystyrene materials exhibit high inertness towards irradiation treatment and did not form a significant increase in migration potential.

Table III. Results of the Quantification of Extracts of Polystyrene

Substance(R_t [min])	*Concentration [mg kg^{-1}]*		
	Unirradiated PS	*Irradiated PS 8.5 kGy*	*Irradiated PS 23.9 kGy*
oligomer (23.2)	156	157	162
oligomer (36.4	290	283	295
oligomer (36.6)	366	357	389
oligomer (36.9)	765	746	812
oligomer (37.1)	209	204	223

Polypropylene (PP)

Headspace screening of irradiated polypropylene cups (e-beam) gave similar results to the polystyrene's. Representative headspace gas chromatograms for polypropylene are shown in Figure 4. The concentrations of unidentified radiolysis products with retention times between 1.7 min and 3 min with molecular weight <100 g mol^{-1} increase with increased irradiation dose. Except for two specific radiolysis products related to additive degradation, the concentration of other PP related substances are decreasing or could be detected in similar concentrations as compared to the unirradiated reference samples. These two specific radiolysis products could be identified as 1,3-di-*tert*-butylbenzene (R_t = 10.8 min) and 2,4-di-*tert*-butyphenol (R_t = 12.7 min). Both substances are degradation products from Irgafos 168 (*5, 11, 12, 13*). The gas chromatographic screening of the extracts (Figure 5) verifies the results of the headspace screening and the formation of 1,3-di-*tert*-butylbenzene (R_t = 11.7 min) and 2,4-di-*tert*-butylphenol (R_t = 18.6 min). During irradiation under normal atmosphere the antioxidant Irgafos 168 was completely destroyed at a radiation dose of only 8.5 kGy. Main reaction product was the oxidized form of Irgafos 168 (R_t = 50.6 min). As by-products, the above-mentioned radiolysis products 1,3-di-*tert*-butylbenzene and 2,4-di-*tert*-butylphenol are generated. The results of quantification of the radiolysis products are summarized in Table IV.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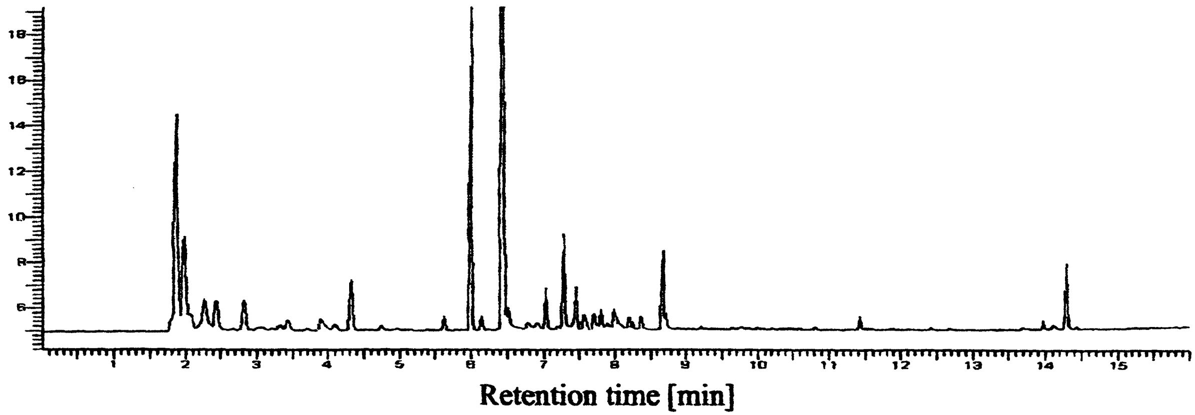
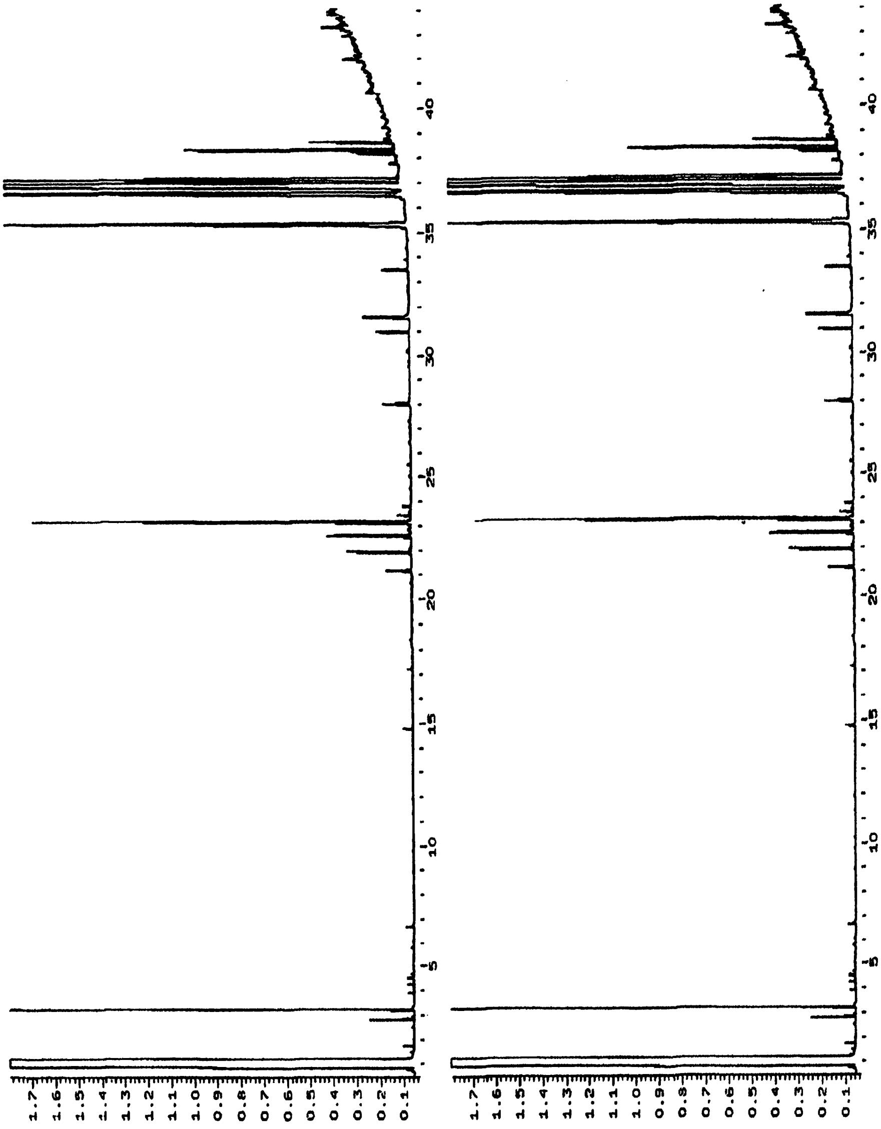


Figure 2. Representative headspace gas chromatograms of PS cups (top: unirradiated reference sample, bottom: irradiated with 23.9 kGy)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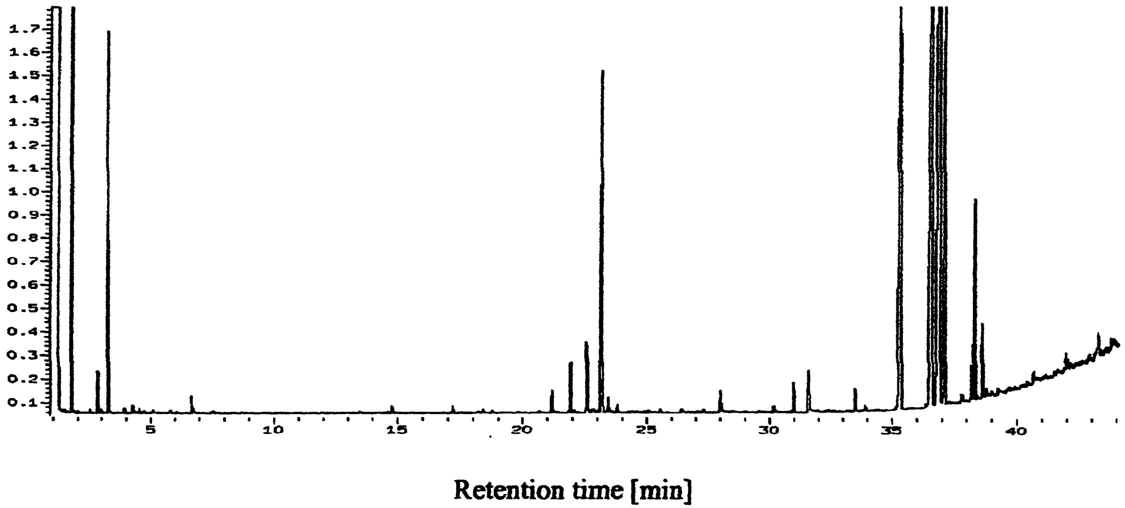


Figure 3: Representative gas chromatograms of the extracts of PS cups (top: unirradiated reference sample, middle: irradiated with 8.5 kGy bottom: irradiated with 23.9 kGy)

It should be noted here that due to the complete degradation of Irgafos 168 most probably the protection of the polymer against oxidative stress is lost.

In summary, irradiation increases the concentrations of highly volatile substances and two specific radiolysis products in polypropylene. This is in contrast to the above-mentioned effects of irradiation on PET and PS, where only slight effects are determined. Due to the degradation of Irgafos 168 and the formation of 1,3-di-*tert*-butylbenzene and 2,4-di-*tert*-butylphenol, it is expected that the specific migration behavior of the investigated PP could be changed. In addition, the increase of highly volatile substances may change the sensory properties of the investigated packaging materials.

Low-Density Polyethylene (LDPE)

Representative headspace gas chromatograms of LDPE are shown in Figure 6. The comparison between the unirradiated reference sample and the irradiated LDPE shows that highly volatile compounds occur in the time range between 1.5 and 3.5 min. The other substances, which are identified as oligomers, are not affected by the radiation treatment. A clear identification of these highly volatile substances failed due to the aforementioned reasons. The gas chromatographic screening of the extracts does not show any significant changes in the concentration of substances relevant to migration (Figure 7).

In summary, the investigated LDPE material shows only a slight increase in highly volatile substances after irradiation. In addition, only a slight impact of irradiation on other compounds was detected. In comparison to the internal standards, butylhydroxyanisol (R_t = 17 min) and Tinuvin 324 (R_t = 49 min), the concentration of qualitatively new radiolysis products in the irradiated LDPE samples are far below 50 ppm.

Sensory Evaluations

The influence of irradiation on the sensory behavior was investigated with the PP and PS cups which are intended for packaging dairy products. As shown from the gas chromatographic screening, polypropylene shows an increase in highly volatile substances. In the case of polystyrene a high inertness towards irradiation was detected. Both samples are therefore different in their behavior towards ionizing radiation, and from the results of the gas chromatographic screening it is expected that in the case of polypropylene the increase in volatiles may lead to different sensory properties before and after irradiation whereas polystyrene should result in similar sensory properties.

The results of the sensory evaluation of odor of the irradiated polymers and the taste of filled and stored water are given in Table V and Table VI, respectively. In this study water was used instead of the original foodstuff in

Table IV. Results of the Quantification of Polypropylene (Dichloromethane Extracts)

Substance(R_t [min])	*Concentration [mg kg^{-1}]* *Unirradiated PP*	 *Irradiated PP 8.5 kGy*	 *Irradiated PP 23.9 kGy*
1,3-di-tert-butylbenzene (12.2)	n.d.	11.2	30.4
2,4-di-tert-butylphenol (18.5)	1.6	41.5	59.8
unknown (28.0)	10.8	20.8	21.5
stearic acid (31.6)	21.3	41.0	47.4
Irgafos 168 (48.8)	118	2.5	n.d.
oxidated Irgafos 168 (50.6)	99.4	180	82.2

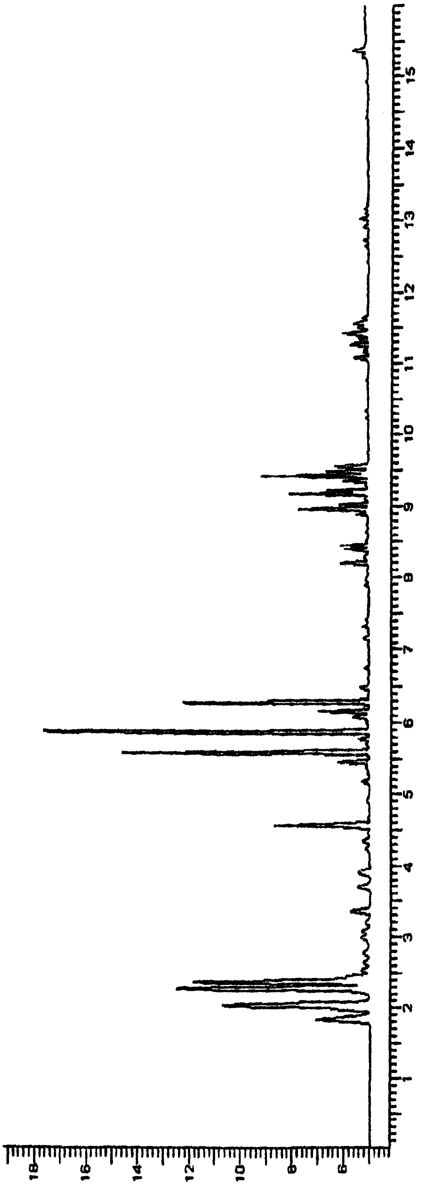
n.d. (not detected)

Table V. Results of the Sensory Evaluation (Odor) of Irradiated Yogurt Cups

Sample dose	*Characterization of odor*	*Intensity of odor*
PS, unirradiated	plastic odor, burnt, slightly pungent, styrene (monomer)	2-3
PS, 8.5 kGy	plastic odor, waxy, sweaty, burnt	3
PS, 23.9 kGy	plastic odor, waxy, fatty, burnt	3
PP, unirradiated	plastic odor, burnt	3-4
PP, 8.5 kGy	plastic odor, spicy, burnt, slightly sweaty	3
PP, 23.9 kGy	sweet plastic odor, spicy, slightly sweaty	3

Table VI. Results of the Sensory Evaluation (Taste of Water) of Irradiated Yogurt Cups

Sample dose	*Characterization of taste*	*Intensity of taste*
PS, unirradiated	slightly plastic odor	threshold
PS, 8.5 kGy	slight plastic taste with slight chemical off-note	1.5
PS, 23.9 kGy	slight plastic taste with slight chemical off-note	1.5
PP, unirradiated	slightly plastic odor	threshold
PP, 8.5 kGy	slightly plastic odor	1.0
PP, 23.9 kGy	slightly plastic odor, burnt	1.5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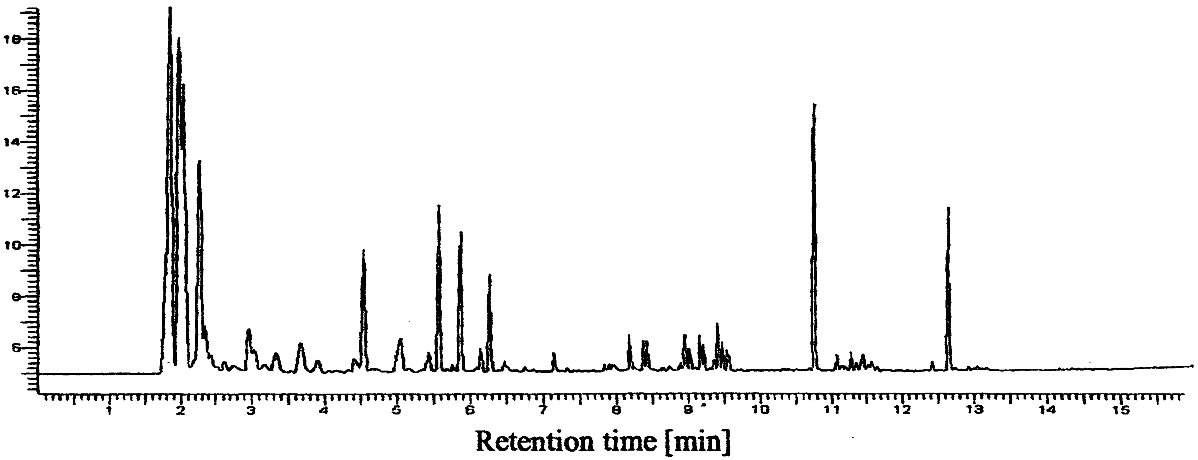
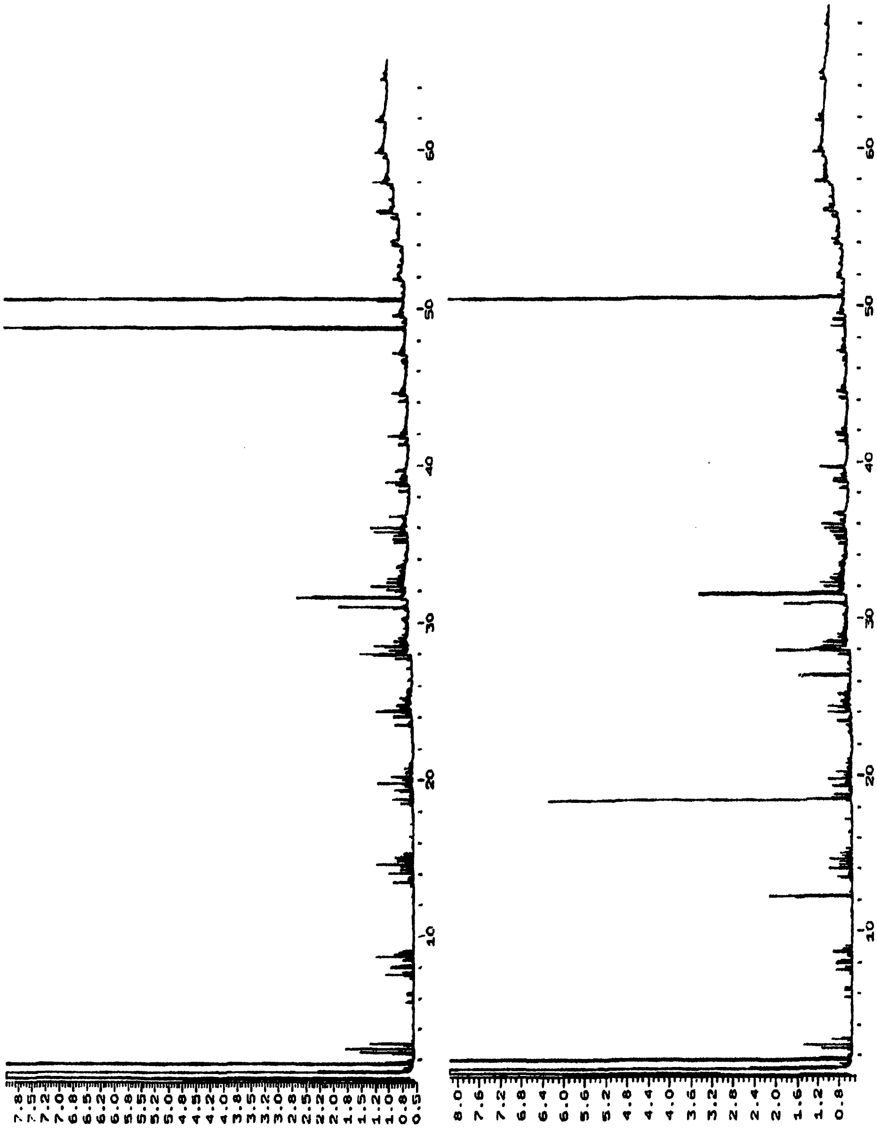


Figure 4. Representative headspace gas chromatograms of PP cups (top: unirradiated reference sample, bottom: irradiated with 23.9 kGy) (Reproduced with permission from reference 13. Copyright 2002.)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40
50
60
7.8
7.5
7.2
7.0
6.8
6.5
6.2
6.0
5.8
5.5
5.2
5.0
4.8
4.5
4.2
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3.5
3.2
3.0
2.8
2.5
2.2
2.0
1.8
1.5
1.2
1.0
0.8
0.5
8.0
7.6
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6.4
6.0
5.6
5.2
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3.2
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0.8

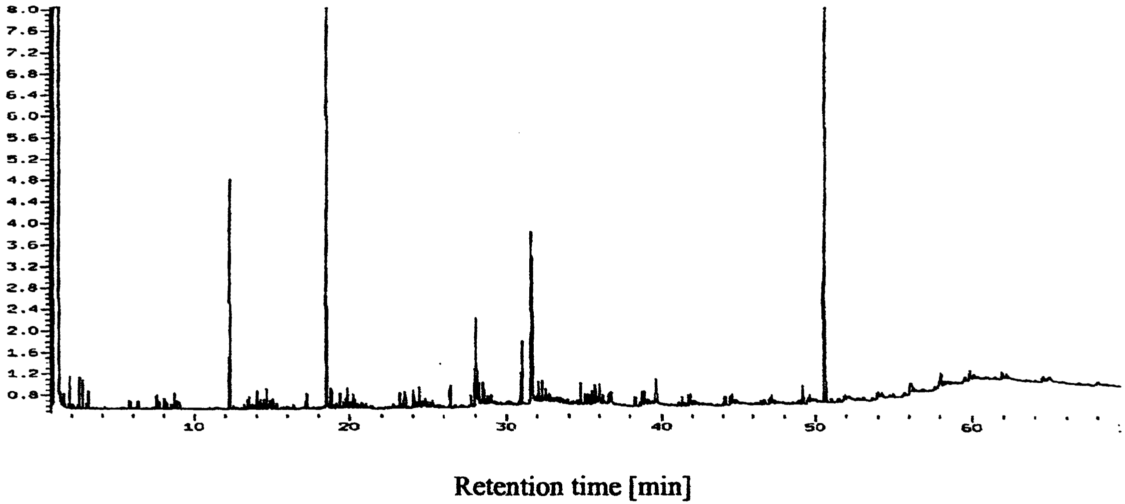
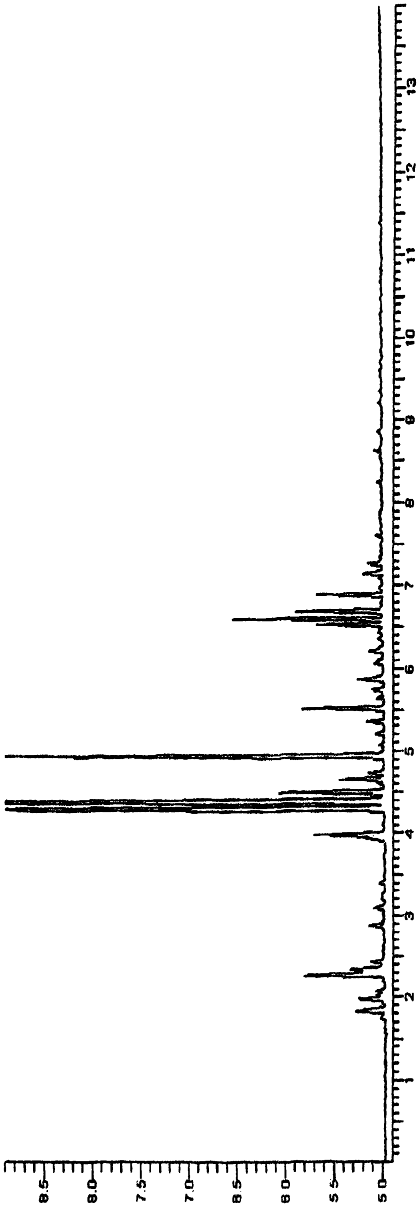


Figure 5. Representative gas chromatograms of the extracts of PP cups (top: unirradiated reference sample, middle: irradiated with 8.5 kGy, bottom: irradiated with 23.9 kGy)


8.5
8.0
7.5
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6.0
5.5
5.0
1
2
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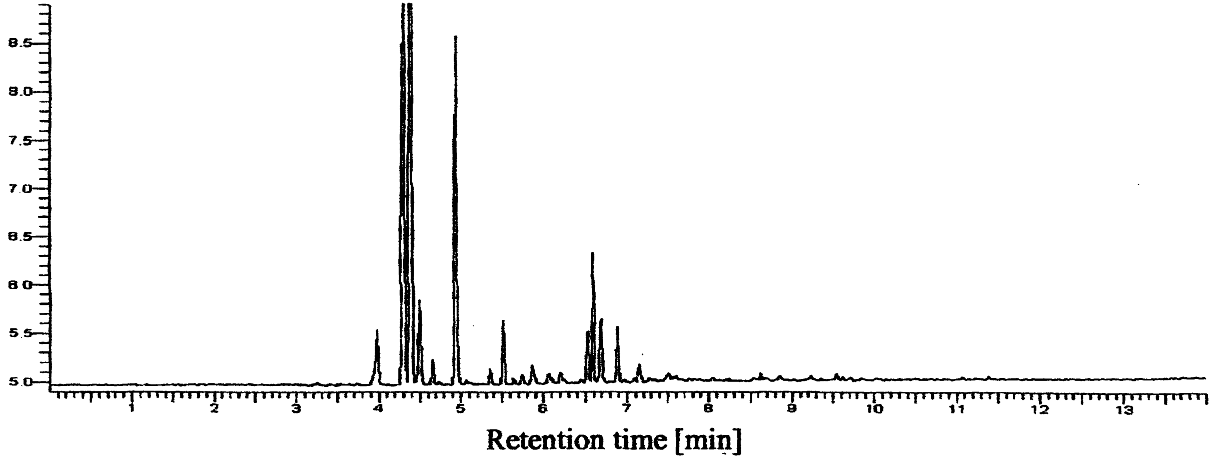
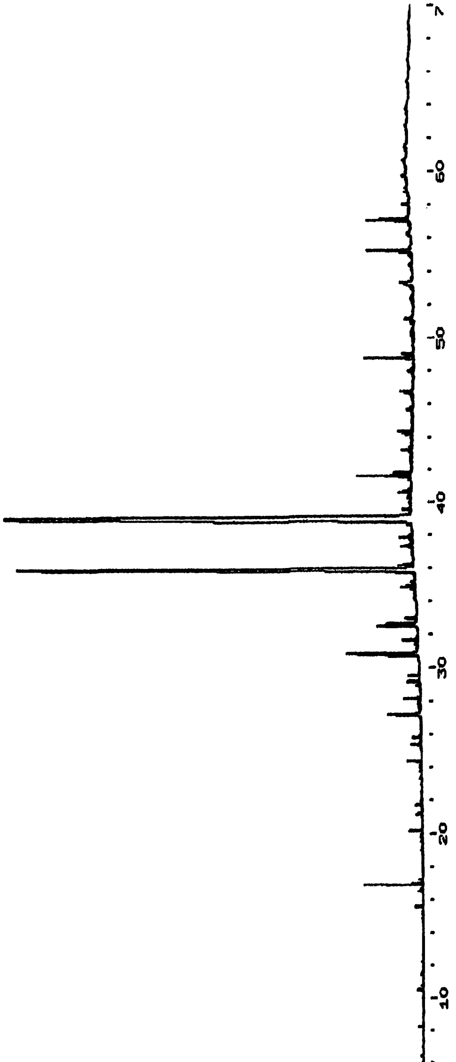


Figure 6. Representative headspace gas chromatogram of LDPE for pharmaceutical packaging (top: unirradiated reference sample, bottom: irradiated with 15 kGy)


2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
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10
20
30
40
50
60
7

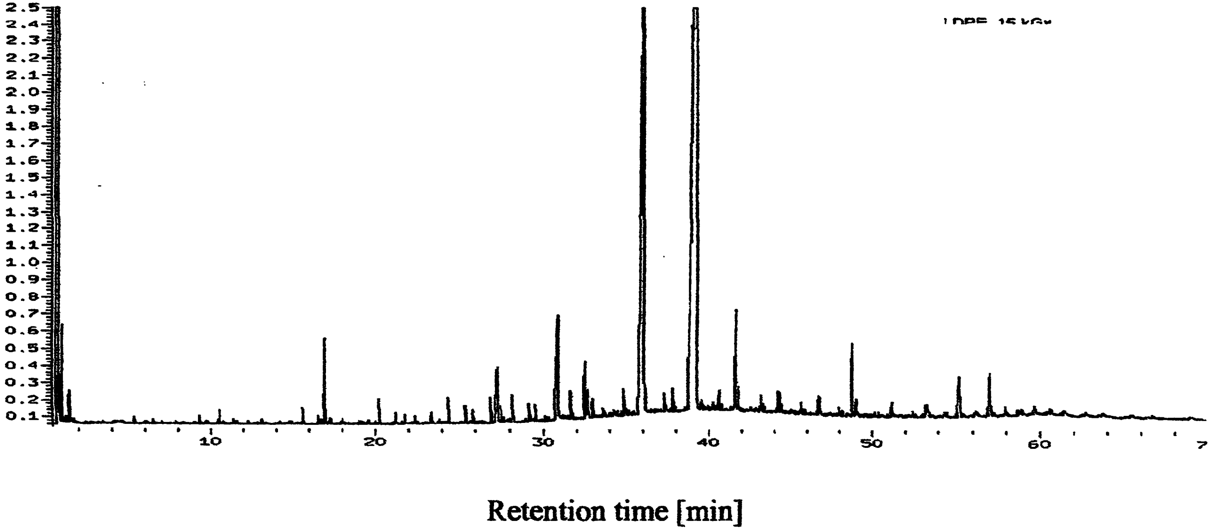

Figure 7. Representative gas chromatogram of LDPE for pharmaceutical packaging (top: unirradiated reference sample, bottom: irradiated with 15 kGy, internal standards butylhydroxyanisol (17 min) and Tinuvin 324 (49 min)

order to have a very sensitive matrix for sensory evaluation. Due to the sterilization with ionizing radiation the PS cups developed a slight off-odor. This was also observed with the irradiated PP cups, however, the developed odor was less intensive compared to the unirradiated PP cups. The sensory results show no significant correlation between the concentration of highly volatile substances and the intensity of detected off-odor, but in any case with increasing radiation dose the odor was described typically with a more unpleasant burnt or sweaty character. On the other hand, the taste of water stored in both cups after irradiation was only slightly influenced by the off-odor.

Migration Considerations

Migration was described in detail in our previous work (*12, 13*). In these studies, the overall migration results of packaging films were not influenced by irradiation even up to a very high irradiation dose of approximately 70 kGy. However, it must be noted that the overall migration test procedure allows a considerable loss of volatile substances during evaporation of food simulants. Therefore, the overall migration test based on gravimetric procedures is not suitable for detection of volatile substances. In view of specific migration due to the effect of irradiation on the antioxidants like Irgafos 168, the migration behavior may change. In our previous work, the specific migration of the additive related compounds (1,3-di-*tert*-butylbenzene and 2,4-di-*tert*-butylphenol) was determined from three different polyolefin packaging films (*12*). However, the resulting concentrations of 1,3-di-*tert*-butylbenzene and 2,4-di-*tert*-butylphenol are in the low ppb range.

In spite of the positive results of the overall migration studies, the migration of volatile radiolysis products into a particular foodstuff cannot generally be excluded. An inherent problem of irradiated polymers is the formation of small molecules with high diffusion potential. The migration potential should be determined case-by-case for any new packaging application and be evaluated with respect to possibly occurring migration levels in a foodstuff.

Conclusions

It can be shown from the results of this study as well as from our previous work (*11, 12, 13*) that irradiation of packaging materials can lead to formation of volatile compounds during irradiation. In most cases irradiation at low doses has only slight effects on the polymer. Typically, concentrations of radiolysis products formed in packaging plastics at relevant doses (5 – 25 kGy) are in the low ppm range, which is similar to technical impurities in polymers. Due to the formation of volatile radiolysis products, however, migration of these compounds cannot be excluded. A general evaluation of the toxicological risk for all packaging materials and additives is very difficult due to the strong dependency of the effects on polymer material, the nature of additives, the additive concentrations and irradiation dose. Migration of radiolysis products (at 10 kGy dose) into food simulants was found at maximum in the low ppb range, close to or below the US FDA threshold of regulation limits.

An evaluation of the food law compliance of the packaging material intended for irradiation should be done under consideration of the applied radiation dose and in comparison to the unirradiated polymer. The most important factor seems to be the sensory properties of irradiated plastics. Therefore sensory testing is highly recommended using applied dose and foodstuff type. In order to safeguard the compliance of the irradiated packaging materials with legal requirements, the major focus must be put on the evaluation of the specific migration potential formed by irradiation. In addition to or even instead of migration tests, we recommend an analytical screening on the irradiated plastics comparative to reference samples with methods, which are suitable for detection of volatile substances e.g. headspace gas chromatography.

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Chapter 16

Effects of Gamma Irradiation on Polyethylene, Polypropylene, and Polystyrene

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The effects of gamma irradiation on different antioxidants and ultraviolet (UV) stabilizers, on commercial products, and on model films and sheets were studied. After 30 kGy only small changes were observed for the antioxidants and UV stabilizers irradiated alone, or for the UV stabilizers blended into polyethylene (PE) sheets. In contrast, the concentration of antioxidants in PE sheets and their migration levels into *n*-heptane decreased drastically. Commercial PE, polypropylene (PP), and polystyrene (PS) products were then investigated at dose levels of 10, 30, and 50 kGy. Volatiles, *e.g.* acetic acid, propionic acid, butanoic acid, pentanoic acid, acetone, were detected in PE and PP products only after irradiation; they should be degradation products of the polymer. The contents of antioxidants decreased significantly, whereas lubricants and a plasticizer decreased only slightly. 1,3-Di-*tert*-butylbenzene and 2, 6-di-*tert*-butyl-1, 4-benzoquinone were found as degradation products of antioxidants. On the other hand, few degradation products were detected with PS. Finally, PE and PP model films, and PS model sheets were prepared with different concentrations and combinations of antioxidants. The PS model sheet was very stable, even without antioxidants. In contrast, the antioxidants in the PE- and PP-films decreased quickly, while their presence reduced the formation of degradation products and the loss of mechanical strength. The results illustrate the important role of antioxidants in the stabilization of polyolefin, and they show that irradiation can produce measurable amounts of degradation products that are not found in non-irradiated materials.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Introduction

Food packaging materials may be exposed to gamma irradiation during the irradiation of prepackaged food, or when packaging materials are sterilized for semi-aseptic packaging. In Japan, food irradiation is only permitted to inhibit the sprouting of potatoes. No approval is needed for the sterilization of packaging materials, which is carried out to improve the shelf-life of food.

Gamma irradiation causes various changes in plastic materials. The main change is the degradation of polymer chains, followed by the generation of degradation products. Ultimately, the strength, color, and odor of the material may be affected (*1-5*).

The addition of antioxidants can effectively suppress the degradation of the polymers, while degradation of the antioxidants themselves can cause color changes and the formation of off-odors. Some studies on antioxidants have been reported (*6-9*), but, overall, the effects of gamma irradiation on polymers with antioxidants have not been investigated comprehensively yet.

The author has studied the effects of gamma irradiation on various antioxidants and UV stabilizers (both in bulk form and blended into PE sheets) (*10)*; on commercial PE, PP, and PS products in contact with food (*11, 12*), and on PE, PP, and PS model films and sheets containing various antioxidants (*13*). In this paper, these studies are reviewed.

Materials and Methods

Chemicals

Standards of additives and degradation products were obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan), Sigma-Aldrich Japan Co. (Tokyo Japan), Wako Pure Chemical Industries (Osaka, Japan) and others.

Materials

PE sheets (0.5 mm thickness) with 27 different antioxidants and UV stabilizers: the additives were divided into 2 groups (cf. Table I), added at 100 µg/g each to PE pellets, and molded to sheets at 160 °C.

Commercial PE products: Pellets without additives (1 sample), bags (5), and wrap films (2)

Commercial PP products: Wrap film for rice ball (1), cup (1), and non-woven fabric sheet (1)

Commercial PS: High-impact PS (HIPS) and PS foam (EPS) disposable cup (1 each)

PE model film (0.025 mm thickness): (A) no additive, (B) Butylhydroxytoluene (BHT) 500 μg/g, (C) BHT 2500 μg/g, (D) BHT 500μg/g, Irganox 1076 1000 μg/g, and Irgafos 168 1000 μg/g

PP model film (0.05 mm thickness): (A) no additive, (B) BHT 500 μg/g, (C) BHT 2500 μg/g, (D) BHT 500 μg/g, Irganox 1010 1000 μg/g, and Irgafos 168 1000 μg/g

PS model sheet (0.25 mm thickness): (A) no additive, (B) BHT 200 μg/g, (C) BHT 1000 μg/g, (D) BHT 200 μg/g and Irganox 1076 500 μg/g

Gamma Irradiation

Samples were put into glass bottles or headspace vials for headspace/GC/MS analysis and sealed. They were irradiated with dose of 0, 10, 30 or 50 kGy at a dose rate of 5 kGy/hr in a 130 TBa ^{60}Co gamma-irradiator. After irradiation, analytical determinations were made as soon as possible.

Instrumental Conditions

1. HPLC

Instrument: LC-6A, SPD-6AV, SCL-6B, Shimadzu Seisakusyo Co., (Kyoto, Japan); column: TSKgel ODS-80Ts (4.6 mm i.d., 250 mm length, 5 μm pore size, ODS C18 phase), Tosoh Co. (Tokyo, Japan); column temperature: 50 °C; initial mobile phase: 60% acetonitrile/water; gradient profile: 60% acetonitrile (at 0 min), then linear to 100% acetonitrile (0-13 min), then held at 100% acetonitrile (13-22 min); flow rate: 1.5 ml/min; detection wavelength: 225 nm; sample size: 10 μl

2. GC/MS

Instrument: HP5890 Series II plus, HP5972 Series, Vectra XM-2, Hewlett Packard Co. (Wilmington, DE)

1) Volatiles

Column: DB-WAX (0.25 mm i.d., 30 m length, 0.25 μm film thickness), J&W Scientific (Folsom, CA); column temperature: 45 °C (5 min) → 20 °C/min → 240 °C (10 min); injection temperature: 200 °C; inlet temperature: 280 °C; carrier gas: He, 1.35 ml/min; scan range: *m/z* 40-700.

2) Additives and other Chemicals

Column: DB-1 (0.25 mm i.d., 5 m length, 0.1 µm film thickness); column temperature: 50 °C → 20 °C/min → 300 °C (10 min); injection temperature: 250 °C; inlet temperature: 280 °C; carrier gas: He, 1 psi (constant pressure); sample size: 1 µl; scan range: *m/z* 40-700.

3. Headspace Sampler (HS)

Instrument: HP-7694, Hewlett Packard Co.; temperature: oven 140 °C (PS 60 °C), sample loop 150 °C (PS 70 °C), transfer line 160 °C (PS 80 °C); heating time: 5 min; injection time 0.5 min; injection volume: 1 ml.

The oven temperature was selected at the highest temperature without thermal decomposition on each non-irradiated polymer.

Analysis of Volatiles

Samples (1.0 g) were weighed into 10-ml headspace vials and sealed with a septum and aluminum cap. After irradiation they were analyzed by HS/GC/MS.

Analysis of Additives and Other Chemicals

Samples were cut into small pieces; 0.5 g polymer was extracted with 10 ml cyclohexane and 2-propanol (1:1) at 37 °C for 16 hr. The extract was filtered, and 5 ml of the filtrate were concentrated to about 0.2 ml with a vacuum concentrator. Then 4.5 ml of acetonitrile were added at ca. 50 °C, mixed thoroughly, and made up to 5.0 ml at room temperature. The solution was filtered with a membrane (pore-size 0.45 µm) and analyzed by HPLC and GC/MS. A detailed description of the extraction method may be found elsewhere (*14*).

Migration Test

Test pieces of 1 cm x 2 cm each (surface area 4 cm^2) were soaked in 8 ml of the following food simulants: water, 20% ethanol, 4% acetic acid, and n-heptane. With the first three (aqueous) simulants, the test was carried out at 60 °C for 30 min, and the test solutions were analyzed by HPLC directly. With *n*-heptane, the test was done at 25 °C for 60 min; the heptane was concentrated to about 0.2 ml, diluted with acetonitrile, and analyzed by HPLC.

Tensile Strength, Color, and Odor Tests

Tensile strength was determined with an Autograph AG-20KNG tensile tester, Shimadzu Seisakusyo Co., (Kyoto, Japan). Color and odor was assessed in a sensory test using a panel of three testers.

Results and Discussion

Antioxidants and UV Stabilizers: Bulk Materials vs. Additives Blended into PE Sheets

Antioxidants and UV stabilizers were irradiated either as bulk materials, or blended into PE sheets. After irradiation with a 30-kGy dose, the additive content was determined by HPLC, and the migration test of PE sheets was carried out.

The antioxidants and UV stabilizers irradiated in bulk were only slightly affected or showed no changes compared to the non-irradiated ones. When the additives were blended into PE, most of antioxidants decreased drastically. Among them, Ionox 100, Yoshinox 425, Ionox 220, Ionox 129, Nonflex CBP and Irgafos 168 disappeared completely. In contrast with the UV stabilizers blended into PE, only slight or moderate changes were observed (Table I). This observation indicates that the antioxidants probably play an important role in stabilizing the PE sheet against gamma-irradiation.

Table I. Additives in PE Sheets Irradiated with 30 kGy: Relative Contents and Migration Levels into *n*-Heptane to the Non-Irradiated Control

Antioxidants	*Relative Content (%)*	*Relative Migration (%)*	*Antioxidants*	*Relative Content (%)*	*Relative Migration (%)*
Ionox 100[b]	< 8.0	< 20.0	Irganox 1330[a]	38.9	< 14.0
Yoshinox SR[a]	18.3	24.6	Irganox 1076[a]	30.0	< 9.0
BHT[a]	46.3	< 16.0	Irgafos 168[a]	< 3.0	< 15.0
Noclizer M-17[b]	30.0	< 18.0	*UV stabilizers*		
Yoshinox 2246R[a]	9.9	< 7.0	Cyasorb UV-24[b]	65.4	39.5
Naugard XL-1[b]	50.7	64.0	Seesorb 101[a]	63.9	21.9
Topanol CA[a]	46.5	46.4	Tinuvin P[b]	51.9	51.7
Yoshinox 425[b]	< 3.0	< 7.0	Seesorb 202[b]	79.8	80.8
Cyanox 1790[a]	60.3	65.0	Cyasorb UV-531[b]	89.8	90.8
Ionox 220[a]	< 3.0	< 7.0	Tinuvin 326[b]	42.6	28.4
Ionox 129[b]	< 3.0	< 4.0	Tinuvin 120[a]	93.7	92.0
Nonflex CBP[a]	< 3.0	< 9.0	Uvitex OB[b]	80.9	70.0
Irganox 3114[a]	80.1	76.9	Tinuvin 327[a]	97.3	102.8
Irganox 1010[a]	18.3	<15.0	Tinuvin 328[b]	70.9	77.0

NOTE: Relative content or migration is the percent ratio of content or migration level at 30 kGy relative to that at 0 kGy. All the values preceded by the less than sign (<) are the detection limits divided by the original concentrations at 0 kGy. "a" and "b" identify the two groups added into PE sheets.

SOURCE: Reproduced from reference 10. Copyright 1998 Food Irradiation, Japan

In the migration test, no additive migrated from both irradiated and non-irradiated sheets into the aqueous food simulants, i.e., water, 4% acetic acid, and 20% ethanol. On the other hand, all antioxidants and stabilizers migrated from the non-irradiated sheet into *n*-heptane. Most of the antioxidants did not migrate from the irradiated PE sheets, although all of the UV stabilizers did (Table I). The irradiation seemed to decrease migration comparatively greater than the additive content in the PE sheet.

Commercial PE, PP and PS Products in Contact with Food

The effects of gamma irradiation on commercial PE, PP and PS products in contact with food were studied. The samples were PE pellets, bags, and wrap films; a PP wrap film for rice-ball, a cup, and a nonwoven fabric sheet; and HIPS and EPS disposable cups. Pieces of the samples were irradiated at 0, 10, 30, or 50 kGy dose levels in sealed glass bottles or HS vials. The volatiles were determined directly from the vials by HS/GC/MS; additives and other chemicals were extracted with cyclohexane and 2-propanol (1:1), and determined by HPLC (antioxidants and UV stabilizers) and GC/MS (others).

1. Volatiles

From the PE products, about 15 kinds of volatiles, such as acids, aldehydes, ketones, and alcohols were detected (Table II). Most of them were detected only

Table II. Representative Volatiles from Commercial PE Products

Group	*Chemical*	*Detection ratio*	*Level (μg/g)*	
			0 kGy	*50 kGy*
Acid	Acetic acid	8/8	nd	1.7-9.8
	Propionic acid	8/8	nd	0.6-1.7
	Butanoic acid	8/8	nd	0.4-1.5
	Pentanoic acid	8/8	nd	0.1-0.4
	2,2'-Dimethylpropionic acid	5/8	nd-1.2	0.8-4.0
Aldehyde	Butanal	6/8	nd	0.2-0.4
Ketone	Acetone	8/8	nd	0.6-13.1
	2-Butanone	7/8	nd	0.5-1.6
Alcohol	1-Butanol	8/8	nd	0.2-2.3
	tert-Butanol	8/8	nd	0.4-5.7

NOTE: PE products were a pellet, 5 plastic bags and 2 wrap films. Detection ratio means detected sample number/total sample number at 50 kGy dose, nd < 0.1 μg/g.
SOURCE: Reproduced from reference 12. Copyright 2000 Food Irradiation, Japan

after irradiation and their concentrations were dose-dependent, so they were thought to be radiation-induced degradation products. The main compounds were acetic acid, propionic acid, and acetone, probably degradation products of the PE polymer.

In contrast, 2,2-dimethylpropionic acid is possibly a degradation product of additives, because it could not be found in the PE pellet, bag-4 and wrap film-2 which contained no or few antioxidants, while it was found at high levels in other five samples after irradiation and it was also found in two of five samples before irradiation. These five samples contained high levels of antioxidants such as Irganox 1010 and Irganox 1076.

From the PP products, more kinds of compounds were found than from the PE products (Table III). The same compounds, such as acetic acid, propionic acid, 2,2-dimethylpropionic acid, acetone, and *tert*-butanol were detected, and many branched compounds, such as 4-methyl-2-pentanone, and 4-hydroxy-4-methyl-2-pentanone were also detected. Moreover, the amounts of volatiles were much higher than those from PE. The nonwoven fabric sheet produced the highest amount of volatiles, and it was presumed that its extremely large surface area facilitated the degradation process.

Table III. Representative Volatiles from Commercial PP Products

Group	*Chemical*	*Detection ratio*	*Level (μg/g)*	
			0 kGy	*50 kGy*
Acid	Acetic acid	3/3	nd	6.2-36.7
	Propionic acid	3/3	nd	0.4-2.5
	Butanoic acid	2/3	nd	0.4-0.8
	Pentanoic acid	3/3	nd	nd-0.3
	2,2'-Dimethylpropionic acid	2/3	nd	4.3-15.7
Ketone	Acetone	3/3	nd	5.8-21.3
	2-Pentanone	3/3	nd	0.3-2.7
	4-Methyl-2-pentanone	3/3	nd	0.4-2.6
	4-Hydroxy-4-methyl-2-pentanone	3/3	nd	0.9-4.0
Alcohol	1-Butanol	2/3	nd	0.1-0.2
	tert-Butanol	3/3	nd	2.2-10.9

NOTE: PP products were a wrap film, a cup and a nonwoven fabric sheet. Detection ratio is detected sample number/total sample number at 50 kGy dose, nd < 0.1 μg/g.
SOURCE: Reproduced from reference 12. Copyright 2000 Food Irradiation, Japan

From the PS products, only a few compounds were detected (Table IV). Styrene and ethylbenzene were present in small amounts and decreased upon irradiation. Acetone and *tert*-butanol from the EPS cup might be degradation products, but they were not found from the HIPS cup.

Table IV. Volatiles from Commercial PS Products

Sample	*Chemical*	*0 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
HIPS cup	Ethylbenzene	0.1	0.1	0.1	nd
	Styrene	0.2	0.1	0.1	nd
EPS cup	Ethylbenzene	0.1	0.1	0.1	nd
	Acetone	nd	nd	0.5	0.5
	tert-Butanol	0.4	0.5	0.8	1.1

NOTE: Units are μg/g, nd < 0.1 μg/g.

SOURCE: Reproduced from reference 12. Copyright 2000 Food Irradiation, Japan

2. Additives and Related Chemicals

The changes in the levels of additives and related chemicals in the commercial PE products are shown in Table V. The chemicals that could be extracted by the solvent and determined by HPLC were mainly additives and their derivatives. The PE pellets contained no additives. Meanwhile, six samples contained antioxidants, such as Irganox 1076, Irganox 1010, Irgafos 168, and BHT, and all of them rapidly decreased due to the irradiation. In the case of wrap film-1, which contained four kinds of antioxidants concurrently, Irgafos 168 decreased more rapidly than others. Bag-3 also lost Irgafos 168 more rapidly than Irganox 1076. Some PE products additionally contained lubricants. Hydrocarbons did not decrease, while oleamide and steramide decreased more slowly than antioxidants. Dibutylphthalate, a plasticizer, also decreased slowly.

2,4-Di-*tert*-butylphenol was found in three products after irradiation. Though it has been reported as a degradation product of Irgafos 168 by gamma irradiation (*8, 9*), it was also found in the non-irradiated bag-2 and film-1, and there was no dose response. Therefore, it is difficult to say that it is formed by irradiation. Moreover, Irgafos 168 could not be found in the non-irradiated bag-2, so the 2,4-di-*tert*-butylphenol probably came from some Irgafos 168 that had disappeared during manufacturing process, or from other compounds. On the other hand, 1,3-di-*tert*-butyl-benzene and 2,6-di-*tert*-butyl-1,4-benzoquinone were detected only in the irradiated samples, thus they should be produced due to the irradiation. The latter was reported as a degradation product of Irganox 1010 by irradiation (*8, 9*), although, based on its structure, it could possibly also be formed from BHT or Irganox 1076.

Table V. Additives and Related Chemicals in Commercial PE Products

Sample	*Chemical*	*0 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
Pellet		nd	nd	nd	nd
Bag-1	Irganox 1076	91	nd	nd	nd
	Oleamide	390	344	126	84
Bag-2	Irganox 1010	44	nd	nd	nd
	Hydrocarbons	362	386	382	374
	2,4-Di-*tert*-butylphenol	48	42	38	38
	1,3-Di-*tert*-butylbenzene	nd	6	10	10
Bag-3	Irganox 1076	869	439	13	15
	Iragafos 168	245	nd	nd	nd
	Oleamide	688	672	164	270
	2,4-Di-*tert*-butylphenol	nd	44	40	40
	1,3-Di-*tert*-butylbenzene	nd	5	6	7
Bag-4	BHT	24	nd	nd	nd
	Steamide	236	244	172	180
Bag-5	BHT	21	nd	nd	nd
	Oleamide	198	152	116	26
	Dibutylphthalate	19	22	15	11
Film-1	BHT	151	48	29	10
	Irganox 1076	499	307	nd	nd
	Irganox 1010	432	353	241	245
	Irgafos 168	168	nd	nd	nd
	Dibutylphthalate	152	125	94	45
	2,4-Di-*tert*-butylphenol	56	100	44	42
	2,6-Di-*tert*-butyl-1,4-benzoquinone	nd	34	60	38
Film-2	Dibutylphthalate	154	164	150	108

NOTE: Units are µg/g, nd < 5 µg/g (BHT, 1,3-di-*tert*-butylbenzene), nd < 10 µg/g (Irganox 1076, Irganox 1010, Irgafos 168), nd < 20 µg/g (others).

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The additives in the commercial PP products (Table VI) were affected in the same manner as those in PE. The antioxidants rapidly decreased and most of them disappeared at dose levels above 10 kGy. Lubricants, such as palmitamide, steramide, oleamide and erucamide, and the plasticizer dibutylphthalate decreased more slowly. 2, 4-Di-*tert*-butylphenol, 2, 6-di-*tert*-butyl-1,4-benzoquinone, 1,3-di-*tert*-butylbenzene, and stearic acid were also found in irradiated PP products, and they were thought to be degradation products of additives. Among them, 2,4-di-*tert*-butylphenol and 2,6-di-*tert*-butyl-1,4-benzoquinone might be intermediates of degradation, because they disappeared again at 50 kGy. Stearic acid might be produced from steramide.

Table VI. Additives and Related Chemicals in Commercial PP Products

Sample	*Chemical*	*0 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
Film	Irganox 1010	210	21	nd	nd
	Oleamide	474	354	102	86
	Erucamide	374	412	224	152
Cup	Irganox 3114	21	nd	nd	nd
	Iraganox 1010	142	12	nd	nd
	Palmitamide	156	198	164	138
	Steamide	1026	1422	1228	836
	Stearic acid	nd	nd	444	394
	Dibutylphthalate	37	20	59	19
	1,3-di-*tert*-butylbenzene	nd	7	10	21
Nonwoven fabric sheet	Irganox 3114	491	339	42	56
	Iraganox 1010	148	37	nd	nd
	Irgafos 168	13	nd	nd	nd
	Dibutylphthalate	27	24	22	5
	2,4-di-*tert*-butylphenol	nd	38	38	nd
	2,6-di-*tert*-butyl-1,4-benzoquinone	nd	44	36	nd
	1,3-di-*tert*-butylbenzene	nd	5	14	28

NOTE: Units are µg/g, nd < 5 µg/g (Irganox 3114, BHT, 1,3-di-*tert*-butylbenzene), nd < 10 µg/g (Irganox 1076, Irganox 1010, Irgafos 168), nd < 20 µg/g (others).
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Table VII. Oligomers in Commercial PS Products

Sample	*Chemical*	*0 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
HIPS cup	Styrene dimer-4	220	210	240	200
	Styrene trimer-1	1320	1300	1370	1160
	Styrene trimer-2	2080	2070	2160	2010
	Styrene trimer-3	4200	4200	4330	4070
	Styrene trimer-4	1150	1100	1310	1090
EPS cup	Styrene dimer-4	30	30	30	30
	Styrene trimer-1	340	350	340	310
	Styrene trimer-2	70	70	70	70
	Styrene trimer-3	180	180	210	190
	Styrene trimer-4	60	60	70	70

NOTE: Units are µg/g, Styrene dimer-4: *trans*-1, 2-diphenylcyclobutane, Trimer-1: 2,4,6-triphenyl-1-hexene-1, Trimer-2 to 4: 1-phenyl-4-(1'-phenylethyl)tetralin isomers
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The chemicals in PS products (Table VII) were very different from those in the PE and PP products. In the HIPS and EPS cups no additives were determined, and only styrene oligomers were found. The amounts of styrene dimer and trimers did not change upon irradiation.

Model Films or Sheets Containing Antioxidants

The effects on PE, PP and PS model films or sheets containing different kinds and levels of antioxidants were studied. The model films and sheets were made with additives as described, and the contents of additives, volatiles and other chemicals, tensile strength, color, and odor were tested after irradiation with doses of 0, 10, 30, and 50 kGy.

1. PE Model Films

The levels of some representative chemicals in the PE model films are shown in Table VIII. As with the commercial PE products described above, the antioxidants decreased upon radiation, and Irgafos 168 disappeared most rapidly in film-D containing three different antioxidants. Of the degradation products of PE polymer, acetic acid, propionic acid, and 2-butanone were produced in all PE films after irradiation. The presence of antioxidants reduced the formation of these volatile degradation products, and the combination of BHT, Irganox 1076, and Irgafos 168 (film-D) was more effective than the same amount of BHT by itself (film-C).

Changes in tensile strength, color and odor are shown in Table IX. In the tensile strength test, there were no breaks, and the maximum load did not change after irradiation. On the other hand, the color of the sample without additives (film-A) was semi-clear, but those with additives were slightly yellowish and became yellow with increasing dose. In the odor test, the sample without additives initially had no odor, and then a burnt and acidic smell at higher doses, while the samples with additives initially had a BHT-like smell, and then a burnt and chemical smell at higher dose levels. Overall, coloration was more pronounced, and odors were stronger in the three films containing antioxidants compared to the non-stabilized film.

2. PP Model Films

Representative chemicals in the PP model films after irradiation are shown in Table X. The behavior of the antioxidants and the generation of degradation products showed the same tendency as the PE model films and the commercial PP products. All the antioxidants decreased upon irradiation, and Irgafos 168 disappeared most quickly among them in film-D. However, the PP films produced more different kinds, and much higher amounts of degradation products than the PE films.

Table VIII. Representative Chemicals in PE Model Films

Film	*Chemical*	*0 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
A	Acetic acid	nd	2.30	13.1	14.3
	Propionic acid	nd	0.40	1.46	1.81
	2-Butanone	nd	0.34	0.74	0.25
B	BHT	132	34	21	7
	Acetic acid	nd	nd	9.75	7.89
	Propionic acid	nd	nd	0.99	0.90
	2-Butanone	nd	nd	0.91	0.47
C	BHT	562	180	37	25
	Acetic acid	nd	nd	4.57	4.48
	Propionic acid	nd	nd	0.53	0.59
	2-Butanone	nd	nd	0.34	0.19
D	BHT	235	51	31	32
	Irganox 1076	804	563	329	36
	Irgafos 168	598	33	6	nd
	Acetic acid	nd	nd	0.30	2.45
	Propionic acid	nd	nd	nd	0.35

NOTE: Units are μg/g, nd < 10 μg/g (Irgafos 168), nd < 0.1 μg/g (others).
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Table IX. Tensile Strength, Color, and Odor of PE Model Films

Factor	*Film*	*0 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
Tensile strength	A	no break	no break	no break	no break
	B	no break	no break	no break	no break
	C	no break	no break	no break	no break
	D	no break	no break	no break	no break
Color	A	semi-clear	semi-clear	semi-clear	semi-clear
	B	yellowish	yellowish	yellowish	light-yellow
	C	yellowish	yellowish	yellowish	light-yellow
	D	semi-clear	yellowish	yellowish	light-yellow
Odor	A	free	free	slight-burnt	burnt-acidic
	B	BHT-like	BHT-like	slight-burnt	chemical
	C	BHT-like	BHT-like	BHT-like	slight-burnt
	D	BHT-like	BHT-like	BHT-like	chemical

SOURCE: Reproduced from reference 13. Copyright 2003 Food Irradiation, Japan

Table X. Representative Chemicals in PP Model Films

Film	*Chemical*	*0 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
A	Acetic acid	nd	39.7	58.2	55.6
	Propionic acid	nd	1.17	1.29	1.26
	2-Butanone	nd	8.13	9.68	5.34
B	BHT	260	81	70	28
	Acetic acid	nd	2.35	24.6	41.8
	Propionic acid	nd	0.32	0.85	1.58
	2-Butanone	nd	nd	2.62	5.75
C	BHT	1329	1042	727	262
	Acetic acid	nd	1.56	15.9	10.6
	Propionic acid	nd	0.26	0.57	0.68
	2-Butanone	nd	nd	0.15	nd
D	BHT	316	132	67	51
	Irganox 1010	767	438	222	15
	Irgafos 168	623	nd	nd	nd
	Acetic acid	nd	0.61	8.92	20.2
	Propionic acid	nd	nd	0.53	1.15
	2-Butanone	nd	nd	0.22	0.98

NOTE: Units are μg/g, nd < 10 μg/g (Irgafos 168), nd < 0.1 μg/g (others).
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Other parameters of the PP model films are shown in Table XI. In the tensile strength test, there were no breaks prior to irradiation, but after irradiation most samples broke except for film-D at 10 and 30 kGy. PP films became fragile upon irradiation, and even a high level of BHT could not suppress the effect, while the combination of BHT, Irganox 1010, and Irgafos 168 was more effective. In the color and odor tests, PP films with additives were less colored and less odor than the PE films after irradiation.

3. PS Model Sheets

Chemicals determined in the PS model sheets are shown in Table XII. The antioxidants decreased with irradiation, but only slowly, and the degradation product acetic acid was formed only in small amounts. The physical parameters were little affected by irradiation. The sheets were all clear in the color test, and they had no or only a faint smell in the odor test. These results showed that PS is very stable against gamma irradiation.

Table XI. Tensile Strength, Color, and Odor of PP Model Films

Factor	*Film*	*0 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
Tensile strength	A	no break	break	break	break
	B	no break	break	break	break
	C	no break	break	break	break
	D	no break	no break	no break	break
Color	A	semi-clear	semi-clear	semi-clear	semi-clear
	B	semi-clear	semi-clear	yellowish	semi-clear
	C	semi-clear	semi-clear	yellowish	yellowish
	D	semi-clear	semi-clear	yellowish	yellowish
Odor	A	free	free	slight-burnt	acidic
	B	BHT-like	free	slight-burnt	acidic
	C	strong-BHT	faint	slight-burnt	acidic
	D	BHT-like	faint	faint	slight-acidic

SOURCE: Reproduced from reference 13. Copyright 2003 Food Irradiation, Japan

Table XII. Chemicals in PS Model Sheets

Sheet	*Chemical*	*0 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
A	Acetic acid	nd	nd	0.08	0.32
B	BHT	127	97	82	63
	Acetic acid	nd	nd	nd	0.02
C	BHT	521	456	442	366
	Acetic acid	nd	nd	nd	0.11
D	BHT	91	79	69	56
	Irganox 1076	2128	1882	1877	1741
	Acetic acid	nd	nd	nd	0.28

Note: Units are μg/g, nd < 0.1 μg/g.

SOURCE: Reproduced from reference 13. Copyright 2003 Food Irradiation, Japan

Conclusion

Judging from the reduction of the antioxidants, the generation of degradation products, and the change of strength, color, and odor, the stability of the polymers can briefly be summarized as PS >> PE > PP. This is in agreement with previous reports (*2, 4*). PS is the most stable and changed only slightly at these irradiation doses. Although PP and PE are not stable, antioxidants can enhance their stability. The addition of antioxidants to the polymers suppressed the formation of degradation products and reduced the loss of mechanical strength due to irradiation, but additives, especially BHT, affected the color and odor of the samples. Irradiation reduced the level of the antioxidants in the polymers; especially Irgafos 168 disappeared very rapidly, confirming a similar observation by Allen et al. (*7*). The quick disappearance of Irgafos 168 indicates that it is an effective scavenger for radicals; thus the antioxidant appears to be an ideal candidate to stabilize polyolefines against ionizing irradiation. However, it should also be noted that irradiation produces measurable concentrations of antioxidant degradation products that are not observed in non-irradiated materials. Clearly, additional work is needed to understand the complex radiation chemistry of polymer-additive systems.

Acknowledgement The author would like to thank Dr. Rainer Buchalla for his assistance in revising this manuscript.

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Chapter 17

Postirradiation Transformation of Additives in Irradiated HDPE Food Packaging Materials: Case Study of Irgafos 168

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This paper discusses the evolution of Irgafos 168 and its corresponding phosphate in gamma irradiated high density polyethylene used for food packaging materials. Long-term changes in the levels of the antioxidants were observed up to 6 months after irradiation. These data point out the important role of post-irradiation aging in assessing the magnitude of indirect additives and their migration from packaging materials into food. Investigation of post-irradiation aging is also relevant for a better understanding of degradation mechanisms taking place in plastics during and after irradiation process. The shelf-life of the material and of the packaged food products should be considered in the risk assessment to ensure quality and safety of irradiated food products and packaging materials.

Published 2004 American Chemical Society

Introduction

Food packaging materials can be affected by ionizing radiation in the course of their sterilization for aseptic filling or in the course of food irradiation processes. The radiation-induced degradation in polyethylene (PE) and other polymers has been known for many years, and several reviews and papers have described in detail the effects of irradiation on their chemical structure and mechanical properties (*1, 2, 3, 4*). From these published data, it can be concluded that for the low doses applied upon food irradiation (usually less than 10 kGy) and packaging material sterilization (25 kGy), the mechanical and structural changes in polyethylene (PE) are of a minor extent and significance. Although there are some concerns regarding post-irradiation oxidation for prosthesis and other type of implants in the field of biomaterials (*4, 5*), and for food applications, these changes do not affect the mechanical performance relevant for the intended use and the shelf-life of the materials (*6*). The primary concern in the application of radiation processing to food packaging materials is the migration of indirect additives that can affect sensory quality of packaged products as well as food safety (*7, 8, 9, 10*). The salient feature in the literature, on the production of volatile compounds from irradiated packaging materials, is that most studies have been focused on measuring the levels of low molecular weight chemicals regardless of the material post-irradiation age (*10, 11, 12*). It was observed from electron spin resonance (ESR) spectra that long-lived radicals may be present for many years after irradiation in ultra-high molecular weight polyethylene (*13, 14*). A review of the literature concerning the evolution of food packaging materials after ionization treatment indicates that very few data are actually available relating to that topic and that only a few published studies looked at this aspect of irradiated food packaging materials. These few studies, however, covered only periods of a few hours or a few days (*15, 16*). The interest of studying the effect of irradiation on phosphite antioxidants comes from the fact that they are commonly used as additives to stabilize polyolefins (PP and PE), which are the most widely used polymers in food packaging materials. Therefore, migration rates of these additives into food should be monitored to ensure the food safety. It was previously reported that these additives and some of their degradation products were identified as migrating chemical species from irradiated food packaging materials (*10, 17*). The present paper reports selected results from different studies undertaken within our research group regarding the effect of time after irradiation on tris (2,4-di-*tert*-butylphenyl) phosphite (Irgafos 168) levels and on the formation of its corresponding phosphate compound in high density polyethylene (HDPE) materials. The investigation on the formation of other conversion products identified in the course of these research activities will be published separately later.

Materials and Methods

Materials

Commercial HDPE food trays (DYNO 528 designed for retort cooking) were obtained from Dynoplast, Norway. Tray samples, excluding the rim for sealing, were ground into powder (0.2 mm) in a Wiley mill. During grinding liquid nitrogen was added continuously to keep the mill operating at low temperature to avoid heat-induced chemical changes. The resulting HDPE powder (3 g samples) was placed in sealed vials for irradiation. Standard samples for HPLC experiments were obtained in the following way: (1) Irgafos 168 (denoted PI, Figure 1) was donated by Ciba Specialty Chemicals, (2) we prepared the corresponding phosphate (denoted PA, Figure 1) by quantitative conversion using an emulsion of hydrogen peroxide (30% in water, Aldrich) with dichrolomethane for 1 hour at room temperature. PI and PA concentrations in trays before and after irradiation were determined directly by transmission Fourier transform infrared spectroscopy (FTIR) on samples cut from trays. This method allowed in situ analyses of these phosphorous compounds, which had well characterized IR absorptions, free of interference with the HDPE's IR spectrum. Because of variations of the PI and PA content in the commercial samples between batches, the pre-irradiation concentrations were established for each sample studies by FTIR and/or liquid chromatography (see below).

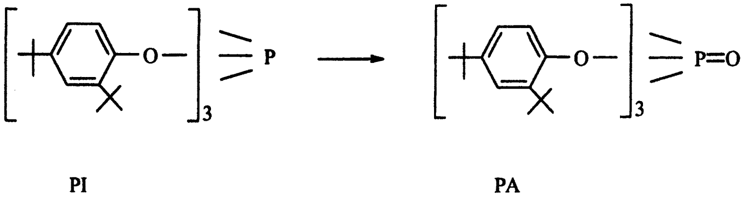

Figure 1. Structure of Irgafos 168 (PI) and its conversion to phosphate (PA)

γ-Irradiation

Vials filled with HDPE samples were irradiated in duplicate by a Nordion 651 PT Gamma beam pilot-scale irradiator (^{60}Co irradiation source, dose rate of 11 kGy/h). Treatment was applied in static air at ambient temperature for absorbed doses of 1, 2, 4, 7, 10, 25, and 48 kGy. The dosimetry was carried out with MSD-Nordion ceric-cerous dosimeters GFSX-197 and F-99 types for dose range of 0-10 and 5-100 kGy, respectively. Dose variation was estimated to be

2%. The irradiated samples were stored in a dark place at ambient temperature prior to extraction.

Soxhlet Extraction

Powder from HDPE samples was extracted for 18h using distilled dicholoromethane solvent in Soxhlet extractors. 0.1 ml of triethylphosphite (TEP) was added into the flasks before extraction to prevent oxidation of antioxidant and radiation-induced degradation products during the work up. Glass beads were added into the flasks in order to keep uniform heating. It was verified that longer extraction time did not result in higher extraction yields. The solutions obtained were vacuum evaporated to dryness and the residues were dissolved in 5 ml of tetrahydrofuran (THF, HPLC grade, Fisher Scientific). One ml of the resultant solution was filtered with syringe filters (0.45 μm, Whatman) and filled into HPLC vials (1 ml, Kimble). HPLC experiments were conducted immediately after the sample preparation. To determine the analyte recoveries from the Sohxlet procedure, the dicholoromethane solvents were spiked with predetermined quantities of PI and PA standards prior to extraction for blank runs (without HDPE powder). The average recoveries for both PI and PA were found to be 87% from HPLC measurements.

Chromatographic Analysis

Chromatographic analyses were performed with a Hewlett-Packard Series 1050 HPLC system equipped with a photodiode array (PDA) detector. A Zorbax C18 reverse phase column was used as a stationary phase. Mobile phase was a gradient combination of acetonitrile, water and THF. The experimental parameters were: mobile-phase flow rate: 1 ml/min; injection volume of the extracts: 20 μl; detector wavelengths: 260 and 280 nm. Based on HPLC chromatograms of standard samples, the retention time for Irgafos 168 (PI) and its corresponding phosphate (PA) were determined to be 24.2 and 23.6 min, respectively. For quantification, a solution with a known concentration was prepared for each standard sample in triplicate. Response factors (units of integrated peak area per microgram) were determined to be 156 for PI at 280 nm, and 118 for PA at 260 nm.

Results

The efficiency of hindered phosphites as polymer stabilizers is due to their effectiveness in decomposing hydroperoxides (*18*). During thermal oxidation and γ-irradiation, the hindered phosphite PI contained in HDPE is considered to be mainly converted to its phosphate form, PA (*18, 19, 20*).

In a previous study on degradation of Irgafos 168 in another batch of DYNO HDPE trays, we used FTIR to measure the concentration-changes from IR absorptions of PI at 848 cm^{-1}, and PA at 965 cm^{-1}, for doses ranging from 0 to 8 kGy. The absorption assignations were confirmed by analysis of the respective pure compounds (*20*).

It is noteworthy that all the FTIR data were obtained within 1 h following irradiation. The data reported in Figure 2 show that the conversion rate of radiation-induced conversion of PI to PA was very significant within a dose range of 0-3 kGy. However, from 3 to 7 kGy, a slight decrease of concentration was observed for both PI and PA. Carlsson *et al.* (*20*) also reported a gradual decrease of PI and a gradual increase of PA during 12 h post-irradiation storage following the irradiation of HDPE to an intermediated dose of 1.3 kGy, where much PI still remained (Figure 2). To the best of our knowledge, this previous work reported for the first time the phosphite loss presumably resulting from long-lived radicals in the polyethylene matrix of commercial food containers. This points out the role of of long-lived radicals in the modification of the concentration profile of the antioxidants during storage of irradiated HDPE food packaging materials. In order to get an overview of this transformation during a storage time closer to reality, we have irradiated the same type of HDPE trays and then estimated the level of PI and PA over a post-irradiation period of 6 months by HPLC analysis of Soxhlet extractives.

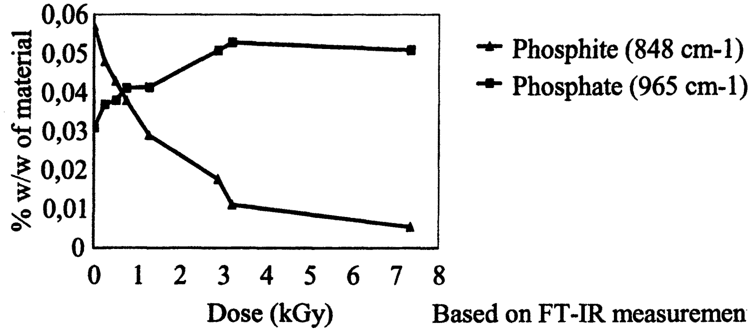

Figure 2. PI to PA conversion in irradiated HDPE trays during irradiation based on FTIR spectral evaluation. (Reproduced with permission from reference 20. Copyright 2001 Taylor & Francis.)

For food irradiation purposes, doses at a lower range of 0.3 - 3 kGy are typically employed to control pests and mold in fresh fruits and vegetables. Doses at a higher range of 25-30 kGy are employed to (1) sterilize packaging materials, (2) treat spices, herbs and dehydrated vegetables or (3) produce ready-to-eat meals (*17*). To cover both of these ranges, approximate doses of 1 and 25 kGy were selected to investigate the effect of post-irradiation storage time on PI and PA. From HPLC measurements, the initial levels of PI and PA in non-irradiated commercial HDPE DYNO trays were estimated to be 407 and 551 ppm, respectively. The presence of PA in all of the non-irradiated trays probably results from oxidative degradation in the course of the extensive mechanical-shear and heating stages, producing free radicals that are involved in the compounding and the molding processes. Additional non-radiation-induced conversion of remaining PI to PA may result from slow in-storage oxidation, within the HDPE resin. All these types of degradation of organic phosphites into phosphates in polymers are well-known phenomena (*19, 21*).

Figure 3 shows HPLC chromatograms demonstrating the PI-to-PA conversion at various room-temperature storage times in HDPE trays irradiated to 1.1 kGy dose. These chromatograms clearly suggest a continuous conversion that is taking place over a period of 6 months.

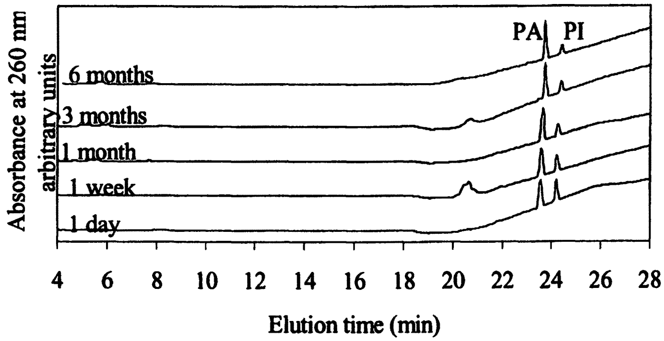

Figure 3. HPLC chromatograms of extractives from HDPE irradiated to 1.1 kGy: Evolution with post-irradiation storage-time at room temperature

Figure 4 shows radiation-induced changes of PI and PA concentrations in HDPE after irradiation at doses of 0, 1.1, and 25.3 kGy as a function of storage time. From these curves, it can be seen that for 0 kGy, the changes of PA and PI concentrations are primarily due to the storage conditions and permeation rate of oxygen through the material. In that case, a very slow linear decrease of the PI concentration was accompanied by a slow, linear increase in the PA concentration. This is clearly shown by the corresponding time-profile of the summation of PI and PA concentrations presented in Figure 5. These concentrations were converted into units of mole/g of material to take into account the fact that PI and PA have different molecular weights, in order to obtain an absolute value of conversion. For 25.3 kGy, no PI was left in the material right after the irradiation (Figure 4). For this dose, only minor changes were observed in the level of PA over 6 months of storage, despite the presence of long-living radicals trapped in the polymer matrix due to the irradiation. Conversely, the time-profile of PA concentrations after a low irradiation dose of 1.1 kGy shows a significant increase during the first month of storage (Figure 4).

Only 12 % of the initial concentration of PI was destroyed during the actual irradiation process (measurement done within less than 24 h after irradiation). But after 6 months, the PI had almost completely disappeared from the material. Both the concentration and production rate of PA in HDPE appeared to be strongly related to the availability of PI in the polymer. The time-profile for the dose of 1.1 kGy shows a significant decrease in the total level of PI and PA over this first month of storage (Figure 5), indicating the existence of additional radiation-induced degradation routes for the PI additive in HDPE following γ-irradiation. The subsequent conversion of PI to PA presents a rate of conversion apparently similar to that in the non-irradiated materials. This observation can be attributed to oxidative degradation reactions that are normally occurring during aging of the material, rather than being related to irradiation effects. It can be deduced from these observations that (1) PA is not affected by long-term radicals but (2) these reactive species (radiation-induced long-lived radicals) affect PI, leading to the formation of degradation products other than PA during the first month of storage.

Simultaneous conversion of PI to chemical compounds other than PA from radiation-induced radicals is probably involved in the course of the irradiation process as well, explaining why the yield of conversion of PI to PA was less than 100% and was dose dependant immediately after irradiation. This hypothesis deduced from HPLC data is in accord with the data previously obtained from the FTIR measurements (*20*), which are presented in Figure 2. This is shown clearly in Figure 6, where the FTIR data are also presented as the summation of the PI and the PA molar concentrations.

These results show that, following irradiation, conversion of PI to PA is less than quantitative and gradually decreases with dose. This important finding is in good agreement with data obtained by HPLC covering the same dose range (*22*).

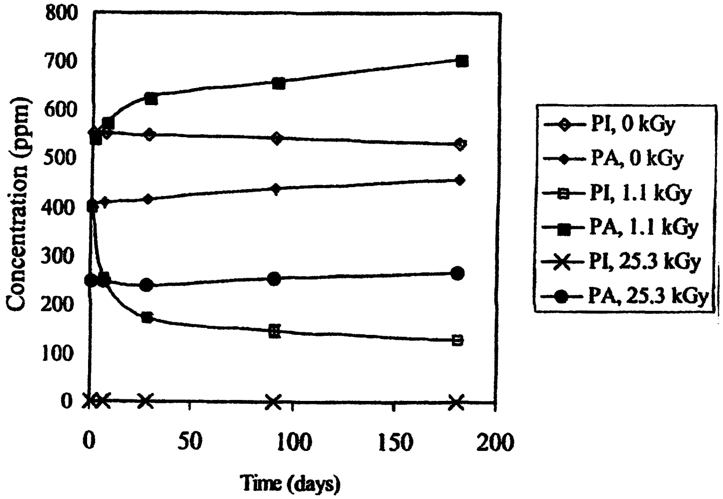

Figure 4. Time-dependence of concentration changes post-irradiation induced in PI and PA in HDPE trays DYNO 528 (from HPLC measurements)

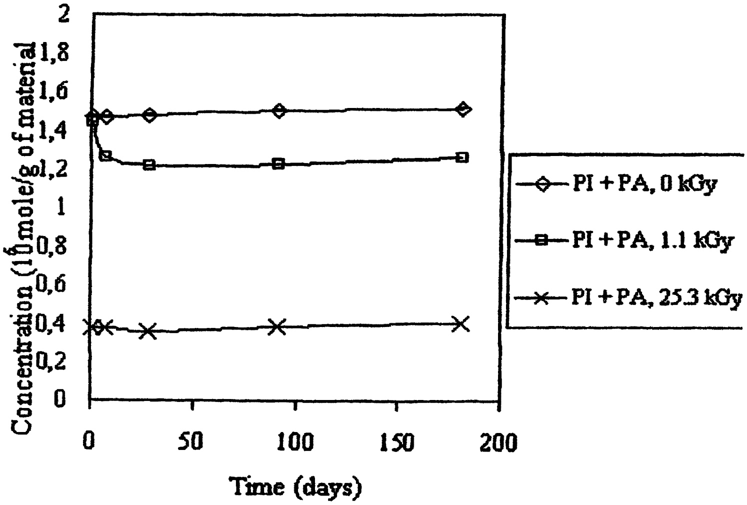

Figure 5. Summation of PI and PA concentrations as a function of storage time (from HPLC measurements)

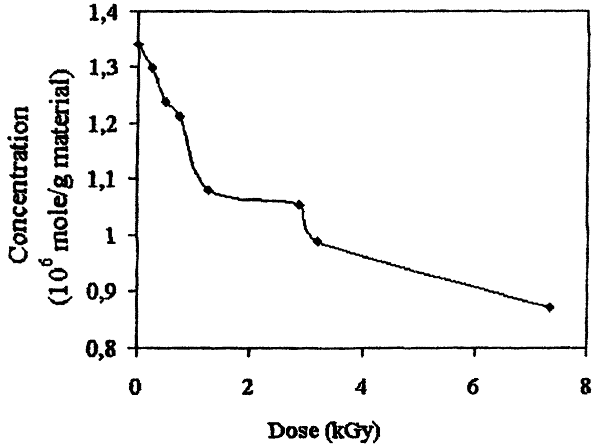

Figure 6. Summation of PI and PA molar concentrations as a function of absorbed dose (calculated from FTIR data obtained less than one hour after irradiation)

From the combined evidence from Figures 4, 5, and 6, we can conclude that the primary PI-to-PA radiation-induced conversion is effected by free radicals and takes place within one month after irradiation. This conclusion is valid for a PI initial level of 407 ppm that has been studied. The results also strongly suggest that subsequent conversion of PI to PA is mostly due to oxidative degradation processes related to normal aging of the material with only minor effect related to radiation-produced, long-lived free radicals.

Discussion

HDPE extraction followed by HPLC analysis for quantification of Irgafos 168 and its corresponding phosphate gave the results that are in good agreement with those obtained from the direct FTIR measurement of these additives in the polymer. As expected, irradiation process induced significant effects on the antioxidant additive. It was observed that the radiation-induced oxidation continues during storage. This can lead to embrittlement of the polymer as already reported for polypropylene articles such as syringes that were radiation-sterilized for medical applications (*23*).

As previously mentioned, from the literature, the hindered phosphite PI contained in HDPE is believed to be mainly converted to its phosphate form, PA, during thermal oxidation and γ-irradiation. Our data presented here indicate that several, radiation-induced, degradation routes for PI are probably taking place simultaneously. On the other hand, it should be kept in mind that the phosphate itself may be degraded in the course of the radiation process; as phosphates are recognized to have a high capture cross-section for the fast electrons, which result from irradiation of the polymer as reported by Halmann (*24*). However, Stevenson and Stein (*25*) mentioned that phosphates do not act as stabilizers in the decomposition of hydroperoxides. From the data generated in the course of the present study, there are indications that the primary degradation of PA takes place during irradiation. This degradation involves species other than the long-lived radicals causing the degradation of the phosphite during post-irradiation storage, or it reflects the difference in the reaction rate of these antioxidants with the remaining radicals. Both PI radiation-induced conversion to chemical species other than PA and PA radiation-induced degradation can explain why the stoichiometric conversion of PI to PA was less than 100% immediately after irradiation and during the post-irradiation storage of the material.

The formation of low molecular weight fragments resulting from degradation of the excited species in irradiated PP, LDPE, and HDPE stabilized with hindered phosphite antioxidants was reported by several research groups (*26, 27, 28*). The identified compounds included 2,4-di-*tert*-butyl-phenol and 1,3-di-*tert*-butyl benzene (the later being considered as a radiation-specific conversion product), resulting from the radiation degradation of the organic phosphites and their phosphate conversion products. These investigations greatly contributed to a better understanding of the transformation of Irgafos 168 in irradiated polyolefins. However, data taking into account the highly significant long-term conversion and fragmentation processes are still lacking in the literature. An ongoing study in our laboratory is focused on measuring the production of 2,4-di-tert-butyl-phenol and 1,3-di-tert-butyl benzene in irradiated HDPE containing Irgafos 168 at a wide range of irradiation doses and post-irradiation storage periods in order to gain a better understanding of the conversion rate profile of PI to PA in the course of γ-irradiation as well as during the subsequent long-term storage.

Last but not the least, we should keep in mind that the migration of small organic fragments from the packaging material into the food is typically faster than that of their organic phosphite and phosphate parents. Hence, it is of great importance to properly study their evolution, as well as the dose and storage-time dependence of their inventory in the packaging, for reliability in assessing the safety of foods packaged therein.

Conclusions

- From both FTIR and HPLC evidences, conversion rate of the radiation-induced phosphite-to-phosphate antioxidants in HDPE was found to be dose and time dependent.

- In-storage conversion takes place primarily within 1 month following irradiation for an initial phosphite level of 407 ppm.
- Radiation-induced phosphite-to-phosphate degradation was found to be accompanied by alternative degradation routes, leading to transformation products other than the corresponding phosphate.
- It would be appropriate to identify all the radiation-induced conversion products of the phosphite, to study their efficiency in stabilizing the polymers of the packaging, and to assess their potential impact on the quality and safety of the packaged food.
- The organic phosphate degradation was found to take place primarily in the course of irradiation, be highly dose dependant, and be insignificantly affected by post-irradiation storage time under the selected experimental conditions.
- In phosphite-stabilized food packaging materials processed by radiation, with or without food content, it is essential to assess the radiation-induced loss of the stabilizer at all dose ranges employed. It is even more essential to assess the radiation-affected inventory of the stabilizer and all its degradation products in the packaging material, at all the dose ranges employed, to ensure the sustained quality of the food packaged therein.

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October 23, 2009 | http://pubs.acs.org
Publication Date: January 2, 2004 | doi: 10.1021/bk-2004-0875.ch018

Chapter 18

Effect of Electron Beam and Gamma Radiation on the Migration of Plasticizers from Flexible Food Packaging Materials into Foods and Food Simulants

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Migration tests were performed using a poly(vinyl chloride) (PVC) film containing di-(2-ethylhexyl)-adipate (DEHA), and a poly(vinylidenechloride-vinylchloride) (P(VDC/VC)) film containing acetyltributyl citrate (ATBC). The PVC-film was gamma-irradiated with 4 and 9 kGy doses while being in contact with either chicken meat samples or food simulant olive oil. Both PVC-and P(VDC/VC)-films were electron beam (EB)-irradiated with 20 and 50 kGy doses while in contact with only olive oil. At the doses studied, the low-dose gamma radiation had no effect on the migration of DEHA from PVC films into the chicken meat and the olive oil. However, the high-dose EB radiation increased migration of DEHA from PVC into the olive oil with the increased dose. In the case of ATBC migrating from P(VDC/VC), an overall migration was much lower than that of DEHA from PVC. Migration of ATBC into olive oil was not affected after 20 kGy and slightly increased after 50 kGy. The results are discussed in relation to EU upper limit for global migration and proposed upper limit for specific migration.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Introduction

Irradiation with ionizing radiation (γ-radiation, electron beam radiation), as a method for food preservation, has been explored and documented over the past 45 years. γ- and EB-radiation have been successfully used for the preservation of foods (poultry, meat, fish, fruits and vegetables) at various dose levels corresponding to "cold pasteurization" and "cold sterilization" (*1-5*).

Since foods are usually prepackaged and then irradiated to avoid microbial recontamination, an important issue is the probable effect of radiation on plastic packaging materials and its consequences, if any, as well as its effect on the quality and/or safety of packaged foodstuffs.

The major changes that are produced in polymers by ionizing radiation are (a) scission and cross-linking of the polymer chains, (b) formation of gases and low-molecular weight volatile radiolysis products, and (c) formation of unsaturated bonds, free radicals and oxidative degradation products in the presence of oxygen. The results depend on the type of polymer, the specific polymer additives used, and the irradiation conditions (*6-13*).

A major concern for foodstuffs prepackaged in plastics is the potential migration of plastics additives such as plasticizers, stabilizers, antioxidants, residual monomers and etc., from the plastic packaging material into the contained foodstuffs (*14-16*). This concern becomes greater in the case of irradiated prepackaged foodstuffs.

One of the main additives of several commercial plastics are plasticizers added to the polymer resin to improve properties such us flexibility, elasticity, and processibility (*16-19*). Plasticizers are oily molecules of low molecular weight having the tendency to migrate into packaged foods, mainly those with high fat content (*17, 18, 20-24*).

There are many studies published in the literature on the toxicity of plasticizers, mainly di(2-ethylhexyl) phthalate (DEHP) (*17, 18, 25-27*). Indications on the toxicity of di(2-ethylhexyl) adipate (DEHA) do exist (*18, 20, 28, 29*) (such as hepatic peroxisome proliferation, infertility and etc. in rats) but the toxicity data for acetyl tributyl citrate (ATBC) are inadequate and currently lacking (*18, 29, 30*). Literature information indicate that ATBC is not a potent multi-site carcinogen, but the induction of a low incidence of a site-specific effect cannot be excluded. High concentrations of ATBC caused a decrease in body weight (bw) and an increase in liver weight in male rats (*29*). At doses corresponding to less than 300 mg/kg bw/day, however, no adverse effects have been recorded (*29*).

Data on the effects of ionizing radiation on global and specific migration of various additives (antioxidants, stabilizers) from polymers to packaged foods have been published in the literature (*7, 8, 15, 31*). However, migration data for plasticizers after exposure to radiation have been limited.

The objectives of the present work were to study the effect of: (a) low and intermediate doses (4 and 9 kGy) of γ-radiation on the migration of DEHA plasticizer from a food grade PVC film into prepackaged foods (chicken meat with and without skin) and a food simulant olive oil and (b) high doses at 20 and 50 kGy of EB radiation on the migration of DEHA and ATBC plasticizers from PVC and P(VDC/VC) films respectively into the olive oil.

Materials and Methods

Materials. The PVC film used was MX-B LM, 15 μm in thickness, and contained 28.3% DEHA (Borden, Chemical Division, MA, USA). The P(VDC/VC) film used was commercial Saran wrap, 15 μm in thickness, and contained 5.0% ATBC (Dow, IN, USA). Analytical grade DEHA was purchased from Fluka (Buchs, Switzerland). Analytical grade ATBC was purchased from Unitex Chemical (NC, USA). Analytical grade 2-ethyl-1-hexanol and 1-butanol were purchased from Merck (Darmstadt, Germany). Refined olive oil was purchased locally and was used as a fatty food simulant in accordance with the EU Directive 97/48/EC (*32*). Unpackaged whole chicken samples were purchased locally and transferred under refrigeration to the laboratory where they were packaged and irradiated.

Irradiation and Migration Experiments

Gamma radiation. Rectangular strips of PVC film (area 460 cm^2) were brought into single-sided contact with 460 ml olive oil as follows: each PVC strip (23 cm x 20 cm) was wrapped around a stainless-steel screen (23 cm x 10 cm) and thermosealed. The film/screen combination was then wound and submerged in a wide-mouthed glass jar (7.5 cm D x 14 cm H) containing 460 ml olive oil and the jar was sealed with a screw cap. The jars were subsequently irradiated with a [^{60}Co] source so as to achieve 4 kGy and 9 kGy doses for each of the two batches irradiated. Irradiation was carried out at 4 - 5°C at a dose rate of 0.6 kGy/h and 1.3 kGy/h for the 4 and 9 kGy doses, respectively. Irradiation doses were measured using Amber Perspex Dosimeters type 3042A. Immediately after irradiation (7 h), samples of contaminated oil were collected for DEHA analysis, and the jars were refrigerated (4 - 5°C) for further sampling at the predetermined time intervals until 97 h (app. 4 days). All experiments were carried out in triplicate. For comparison purposes, identical non-irradiated (control) samples were also analyzed for DEHA content.

Whole chicken meat samples with skin (CMWS) and respective samples without skin (CMWOS) were placed in polystyrene trays (25 cm x 15 cm) and were wrapped with PVC "cling" films. The film/chicken contact area was approximately 400 cm^2 for each sample. The samples were subsequently irradiated with a [^{60}Co] source at an appropriate distance from the source so as to achieve 4 kGy or 9 kGy dose for each of the two batches irradiated. Irradiation was carried out at 8 - 10°C at a dose rate of 1.6 kGy/h. Irradiation doses were measured using the same dosimeters as above.

Irradiation was carried out at the Institute of Material Science, Demokritos Research Center, Athens, Greece. Immediately after irradiation (5.5 h), chicken meat samples (dimensions: 9 cm x 9 cm x 1 cm) were collected for analysis while the rest of the samples were refrigerated for further sampling at the predetermined time intervals until 240 h (10 days). All experiments were carried out in triplicate. For purposes of comparison, identical non-irradiated (control) samples were analyzed for DEHA content.

Electron beam radiation. Circular pieces of PVC and P(VDC/VC) films (area 95 cm^2) were brought into two-side contact (total area 190 cm^2) with 105 ml of olive oil in a glass petri dish. The samples were EB-irradiated at 0 - 2°C with a dose rate of 7.4 kGy/min and 7.9 kGy/min for 20 kGy and 50 kGy doses, respectively. The samples were subsequently stored at 18-20°C until analysis. Irradiation was carried out at the Federal Research Centre for Nutrition, Karlsruhe, Germany. Samples of contaminated oil were collected for plasticizer analysis at intervals between 1 and 288 h. All experiments were carried out in triplicate. For comparison purposes, identical non-irradiated (control) samples were also analyzed for DEHA and ATBC content.

Analytical methods for plasticizers. DEHA and ATBC were determined after saponification and collection of the respective alcohol by steam distillation. Alcohols were then quantified by GC. It should be noted that plasticizer migration was determined indirectly by determining the resultant alcohol formed after hydrolysis. However, it is possible that the plasticizer suffers from radiation-induced transformation and thus the alcohol measurement cannot be attributed from the intact plasticizer alone. Details of the DEHA and ATBC determination methods are given elsewhere (*33-34*).

Results and Discussion

Migration of DEHA into Olive Oil and Chicken Meat after γ-Radiation

a) Olive Oil

An average recovery of DEHA from olive oil was 71.2%. The amounts of DEHA migrating from irradiated and non-irradiated samples into olive oil at 4 - 5°C as a function of time are presented in Tables I-III.

Table I. Migration of DEHA from Irradiated PVC Films (9 kGy) into Olive Oil

Contact time (h)	*Migrated amount of DEHA (mg/l)*	*(mg/dm²)*	*Loss of DEHA from PVC (%)*
7	190 ± 8	19 ± 1	32 ± 1
18	239 ± 10	24 ± 1	41± 2
29	285 ± 12	29 ± 1	48 ± 2
47	313 ± 19	31± 2	53 ± 3
72	297 ± 14	30 ± 1	50 ± 2
97	315 ± 17	32 ± 2	53 ± 3

Data are mean values of triplicate runs ± standard deviation. SOURCE: Reproduced with permission from reference 33. Copyright 1995 Springer-Verlag

Table II. Migration of DEHA from Irradiated PVC Films (4 kGy) into Olive Oil

Contact time (h)	*Migrated amount of DEHA (mg/l)*	*(mg/dm²)*	*Loss of DEHA from PVC (%)*
7	162 ± 7	16 ± 1	28 ± 1
18	268 ± 18	27 ± 2	45 ± 3
29	301 ± 16	30 ± 2	51 ± 3
47	296 ± 12	30 ± 1	50 ± 2
72	312 ± 12	31 ± 1	53 ± 2
97	301 ± 14	30 ± 1	51 ± 2

Data are mean values of triplicate runs ± standard deviation. SOURCE: Reproduced with permission from reference 33. Copyright 1995 Springer-Verlag

Table III. Migration of DEHA from Non-irradiated PVC Films into Olive Oil

Contact time (h)	*Migrated amount of DEHA (mg/l)*	*(mg/dm²)*	*Loss of DEHA from PVC (%)*
1	79 ± 7	8 ± 1	13 ± 1
3	139 ± 7	14 ± 1	24 ± 1
5	176 ± 8	18 ± 1	30 ± 1
7	198 ± 20	20 ± 2	34 ± 3
18	259 ± 10	26 ± 1	44 ± 2
29	279 ± 22	28 ± 2	47 ± 4
47	302 ± 14	30 ± 1	51 ± 2
72	294 ± 21	29 ± 2	50 ± 4
97	295 ± 15	30 ± 2	50 ± 3

Data are mean values of triplicate runs ± standard deviation. SOURCE: Reproduced with permission from reference 33. Copyright 1995 Springer-Verlag

For the non-irradiated (control) samples, the first sampling was initiated 1 h following contact between PVC and oil, instead of 7 h of contact for the first sampling for the irradiated samples.

Tables I-III show that there were no statistically significant differences in the migrated amounts of DEHA between irradiated and non-irradiated samples at equilibrium. Equilibrium conditions were attained after approximately 47 h of contact. The amount of DEHA at the equilibrium conditions was approximately 303 mg/l (30 mg/dm^2), corresponding to a loss of 51%. It is noted that DEHA readily migrated into olive oil when PVC film was brought into contact with this food simulant. After only 1 h of contact at 4 - 5°C, the amount of DEHA that migrated into olive oil was 79 mg/l (8 mg/dm^2), a value corresponding to a loss of 13 % DEHA from PVC. These values are significantly higher than the upper limit of 10 mg/dm^2 (or 60 mg/l) for global migration from plastic packaging materials into food simulants set by the EU (*35*). In all cases, they are also higher than the proposed upper limit of 18 mg/kg for specific migration of DEHA (*36*).

b) Chicken Meat

An average recovery of DEHA from chicken meat was 73.1%. The amounts of DEHA migrating from irradiated and non-irradiated samples into CMWS and CMWOS samples stored at 4 - 5°C for up 240 h were plotted and they are shown in Figures 1-3.

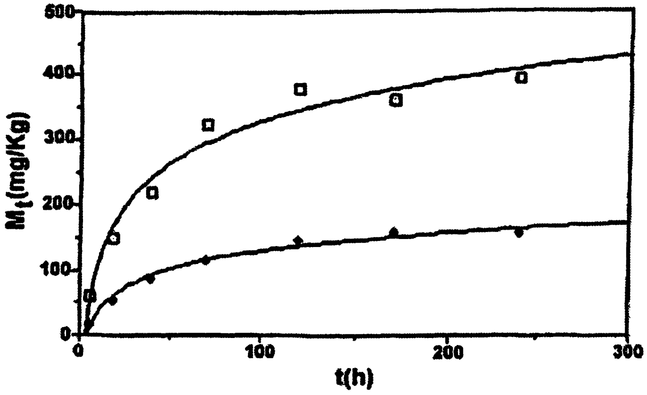

Figure 1. Migration (M_t) of DEHA from 9kGy- irradiated PVC film into chicken meat at 4 - 5°C as a function of time. (□) Chicken meat with skin, (♦) Chicken meat without skin. Relative SD: 3 - 13%. (Reproduced with permission from reference 34. Copyright 1996 Springer-Verlag.)

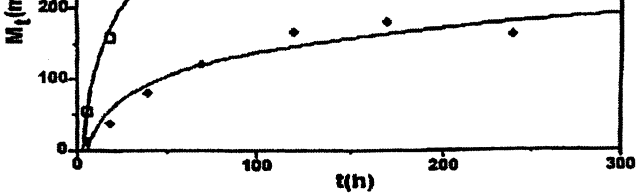

Figure 2. Migration (M_t) of DEHA from 4 kGy- irradiated PVC film into chicken meat at 4 - 5°C as a function of time. (□) Chicken meat with skin, (♦) Chicken meat without skin. Relative SD for the migration data: 5 -12%. (Reproduced with permission from reference 34. Copyright 1996 Springer-Verlag.)

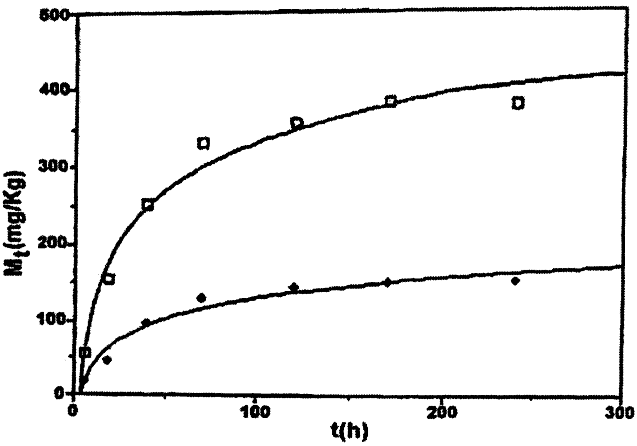

Figure 3. Migration (M_t) of DEHA from non-irradiated PVC film into chicken meat at 4 - 5°C as a function of time. (□) Chicken meat with skin, (♦) Chicken meat without skin. Relative SD for the migration data: 4 - 15%. (Reproduced with permission from reference 34. Copyright 1996 Springer-Verlag.)

Figures 1-3 show that there were no statistically significant differences in the amounts of migrating DEHA between irradiated and non-irradiated samples at equilibrium. There were no differences between samples irradiated at 4 kGy and 9 kGy doses either. These findings are supported by identical IR spectra recorded for the respective PVC samples (data not shown).

It is noted that irradiation does not affect the migration of DEHA into chicken meat (Figures 1-2 vs. Figure 3), but the fat content of the meat sample does drastically (Figures 1-3). The CMWS samples contain fat of approximately 60% while the CMWOS samples contain approximately 7%. After 10 days of contact of chicken meat with PVC at 4 - 5°C, the amount of DEHA migrated into the CMWS samples was 22 mg/dm^2 (396 mg/kg), while the respective amount for the CMWOS samples was 9 mg/dm^2 (158 mg/kg). This large increase in the amount of migrated DEHA (i. e. by a factor of 2.5) is possibly due to the large difference in the samples' fat content and the oily nature of DEHA (*21, 33, 37*).

The measured value of 22 mg/dm^2 for migration of DEHA into the CMWS samples is much higher than the upper limit for global migration from plastic packaging materials into food/food simulant, which is set by the EU at 10 mg/dm^2 or 60 mg/l (*35*). The respective value of 9 mg/dm^2 for the CMWOS samples is, however, within the EU limit for global migration. Nonetheless, both sets of migration values still exceed the proposed upper limit for specific migration for DEHA (18 mg/kg).

The insignificant differences between irradiated and non-irradiated samples under the experimental conditions may be explained by (i) the low rate of irradiation (1.6 kGy/h) and (ii) the small thickness of the PVC film (15 μm). The degree of absorption of radiant energy by a material is given by equation 1:

$$I=I_0e^{-\mu x} \qquad (1)$$

Where: I_0 is the intensity of the incident radiant energy, I is the intensity of the transmitted radiant energy, x is the thickness of the material and μ is the linear coefficient of absorption (*38*). Theoretically, when $x \rightarrow 0$ then $I \rightarrow I_0$, meaning that there is a lower degree of interaction of radiant energy with the material. As the film's thickness decreases the amount of absorbed radiation energy decreases.

A comparison between the present data and those of the PVC/olive oil system shows that at/or near equilibrium the loss of the plasticizer is 51% in the PVC/olive oil system, equivalent to 36% loss in the PVC/CMWS, and 14% loss in the PVC/CMWOS. The lower loss of the plasticizer from the PVC film is anticipated because the film contacts food containing lower fat content like CMWOS.

Till et al. (*21*) reported a value of 19 mg/dm^2 for DEHA migrating from PVC film containing 24 % (w/w) DEHA into chicken breast meat with skin after 7 days of contact at 4°C. This value is in good agreement with our value of 21 mg/dm^2 for DEHA migrating from PVC containing 28.3% DEHA into CMWS samples under the same contact time and the same temperature.

Daun and Gilbert (*39*) reported a value of 23.5 mg/dm^2 for DEHA migrating from PVC film containing 30% DEHA into beef containing 90% fat after 72 h (3 days) at 4°C. This value is comparable to our value of 21 mg/dm^2 after 7 days of contact, given that fat content of the CMWS sample was approximately 60%.

Startin et al. (*40*) reported a value of 5.4 mg/dm^2 for DEHA migrating from PVC film containing 17.2% DEHA into ready cooked meats containing 5% fat after 7 days of contact at 5°C. This value is also in good agreement with our value of 8.6 mg/dm^2 for DEHA migrating from PVC containing 28.3% DEHA into the CMWOS sample containing 7% fat after 7 days of contact.

Migration of DEHA into Olive Oil after Electron Beam Irradiation

Figure 4 shows the amounts of DEHA that migrated from non-irradiated PVC and PVC irradiated at 20 and 50 kGy into olive oil at 20°C as a function of sampling time for up to 288 h. Figure 5 shows a detail of the migration values plotted between 1 and 50 h. Figures 4 and 5 show that the amount of DEHA migrating into olive oil increased with increased radiation dose, and with storage time. The differences between the non-irradiated and irradiated became more noticeable in samples collected during the initial periods of film/oil contact (Figure 5). As the storage (sampling) time increased, the system approached equilibrium, making the differences smaller but the differences were still significant.

After 1 h of PVC/oil contact, the amount of DEHA that migrated into olive oil was 183 mg/l for samples irradiated at a 50-kGy dose, 142 mg/l for samples irradiated at a 20-kGy dose, versus 94 mg/l for non-irradiated samples. After 288 h of PVC film/oil contact, the respective values were 430 mg/l, 409 mg/l, and 391 mg/l. The corresponding loss of DEHA after 1 h of contact was 36% for samples irradiated at a 50-kGy dose, 28% for samples irradiated at a 20-kGy dose, and 18% for non-irradiated samples. After 288 h of contact, the respective values were 84%, 80%, and 76%. These migration values are much greater than the upper limit of 60 mg/kg for global migration set by the EU, and the proposed upper limit of 18 mg/kg for specific migration.

These results are different than those obtained from the low-dose radiation, which showed no effect of irradiation on the amounts of DEHA migrating from

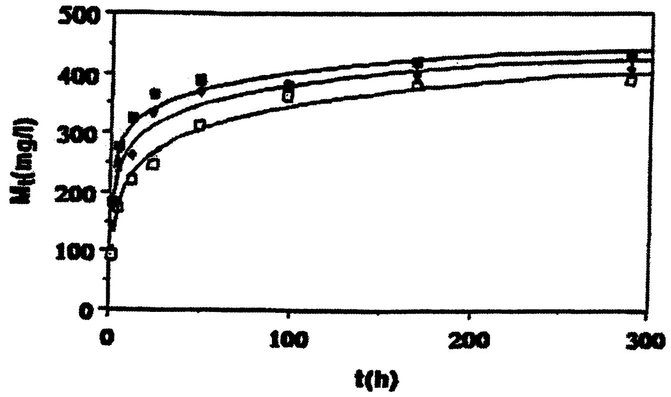

Figure 4. Migration (M_t) of DEHA from PVC films into olive oil at 20°C as a function of time (t) at intervals between 1 h and 288 h. Non-irradiated samples (□), samples irradiated (20 kGy) (♦) and samples irradiated (50 kGy) (■). Relative SD for the migration data: 3 - 10%. (Reproduced with permission from reference 37. Copyright 1998 Int. Assoc. Food Protection.)

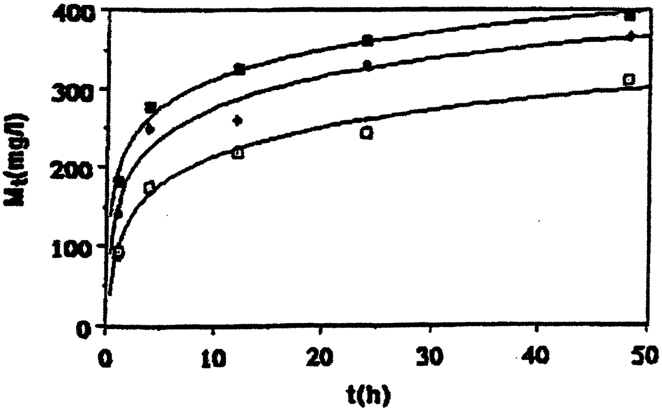

Figure 5. Migration (M_t) of DEHA from PVC films into olive oil at 20°C as a function of time (t) at intervals between 1 h and 48 h. Non-irradiated samples (□), samples irradiated (20 kGy) (♦) and samples irradiated (50 kGy) (■). Relative SD for the migration data: 3 - 8%. (Reproduced with permission from reference 37. Copyright 1998 Int. Assoc. Food Protection.)

PVC film into olive oil and chicken meat samples treated with gamma irradiation at 4 and 9 kGy doses. The differences can be explained by the effects of factors including the type of radiation process (e-beam versus gamma), irradiation dose (20 and 50 kGy versus 4 and 9 kGy), and dose rate (7.4 and 7.9 kGy/min by e-beam versus 0.01 and 0.02 kGy/min by gamma).

It is believed that plasticizers replace polymer-polymer bonds with polymer-plasticizer bonds (*19*). Radiation probably disrupts such polymer-plasticizer bonds resulting in increased migration of plasticizers. The higher the radiation dose, the higher the expected migration level.

The results of this study are in good agreement with those of Killoran (*8*), who reported that e-beam irradiation of plasticized PVC and P(VDC/VC) films at doses in a range of 59-75 kGy increased global migration into n-heptane (fat simulant) after contact at 38°C for 6 weeks. The migrant was mainly an ester-type and an ether-type plasticizer for PVC and P(VDC/VC) films, respectively. Lox et al. (*41*) reported an increase in global migration from PVC shrink films into water after EB-irradiation at a dose of 5 to 25 kGy with a low dose rate (0.6 kGy/h).

Migration of ATBC into Olive Oil after Electron Beam Irradiation

An average recovery of ATBC from olive oil was 63.5%. The amounts of ATBC migrating from non-irradiated P(VDC/VC) films and after 20 and 50 kGy e-beam irradiation into olive oil as a function of time are presented in Table IV. The results show that the migrated amounts of ATBC from non-irradiated samples and samples irradiated with a 20-kGy dose into the olive oil were not significantly different. On the other hand, a 50-kGy irradiation significantly increased migrating amount of ATBC into the olive oil.

Table IV. Migration of ATBC from Non-irradiated P(VDC/VC) Films and after 20 and 50 kGy e-Beam Radiation into Olive Oil at 18- 20°C

Contact	*Migrated amount of ATBC*[a]					
time	*Non-irradiated*		*20 kGy*		*50 kGy*	
(h)	*(mg/l)*	*(mg/dm²)*	*(mg/l)*	*(mg/dm²)*	*(mg/l)*	*(mg/dm²)*
1	ND[b]	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND
12	ND	ND	ND	ND	ND	ND
24	ND	ND	ND	ND	1.6± 0.1	0.08±0.01
48	1.1±0.1	0.06± 0.01	1.3± 0.1	0.07±0.01	2.4± 0.3	0.12±0.02
96	2.3±0.1	0.13± 0.01	2.1± 0.2	0.12±0.01	3.8± 0.3	0.21±0.02
168	3.4±0.2	0.18± 0.01	3.3± 0.1	0.18±0.01	4.0± 0.2	0.22±0.01
288	3.6±0.2	0.20± 0.01	3.6± 0.1	0.20±0.01	4.1± 0.2	0.23±0.01

[a]Data are mean values of triplicate runs ± standard deviation. [b]ND, not detected.
SOURCE: Reproduced with permission from reference 37. Copyright 1998 Int. Assoc. Food Protection

The migrating amount of ATBC was not detectable (<1.0 mg/l) in either non-irradiated samples and samples irradiated at a 20-kGy dose during initial periods of contact for 24 h, versus 12 h for the samples irradiated at a 50 kGy dose. Generally, the migration amounts of ATBC into olive oil were low. After 288 h (12 days) of P(VDC/VC) film-oil contact, the amount of ATBC migrating into olive oil was 3.6 mg/l (0.20 mg/dm²) for non-irradiated samples, 3.6 mg/l (0.20 mg/dm²) for samples irradiated at 20 kGy, and 4.1 mg/l (0.23 mg/dm²) for samples irradiated at 50 kGy. The corresponding losses of ATBC from the films were 3.9%, 3.8%, and 4.4%. These values are well below the upper limit for global migration (60 mg/kg) set by the EU.

It should be mentioned that currently there is no proposed upper limit for ATBC migration (*36*). The amounts of ATBC that migrated into the olive oil are small and may be negligible under normal conditions, given by (1) the low content (5%) of ATBC in the P(VDC/VC) film and (2) the high compatibility (affinity) of ATBC with vinyl resins (*42*).

Conclusions

The results obtained in this study show that low-and intermediate-dose at 4 and 9 kGy, corresponding to “cold pasteurization” of foodstuffs, did not affect

PVC films determined by the specific migration behaviour. This may be explained by (1) the low dose rate of irradiation, (2) the low temperature (8-10°C) of sample during irradiation and the low temperature of sample during storage (4-5°C).

At the higher doses tested, corresponding to "cold sterilization" of foodstuffs, a 20-kGy dose affected only the migration of DEHA from PVC films into olive oil, while a 50-kGy dose affected the migration of both DEHA and ATBC plasticizers from PVC and P(VDC/VC) films, respectively, into olive oil.

A low dose at 4 kGy that was used in this study may be applied (1) with the emerging trend for "minimally processed" food products and (2) for retention of desirable organoleptic characters (odor, taste, color) of foods.

Based on the results in this study, the high migration levels of DEHA plasticizer could become a safety concern. Therefore, the PVC films containing DEHA is not suggested for packaging a fatty food, regardless of whether the food is irradiated or not. On the other hand, both non-irradiated and irradiated P(VDC/VC) films exhibit low migration levels of ATBC plasticizer into olive oil food siumlant. Since ATBC is less toxic than DEHA (*18, 28, 29, 30*), these films may be used without adverse effects for packaging foodstuffs with a high fat content.

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Chapter 19

Physical Evaluation of High-Dose Irradiated Multilayer Pouches

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High-dose irradiated shelf-stable entrees, prepared by the U.S. Army Soldier Systems Command-Natick for NASA, have a formal FDA approval which includes a waiver for packaging material. The total entree process includes filling, vacuum evacuation/sealing, insertion into paperboard cartons, dry ice freezing and shipping, irradiation, thawing and return shipment. In order to minimize package failure five candidate pouches were evaluated including the current "Quad" pouch used for Meal, Ready to Eat (MRE) entrees. Testing included seal strength, leak tests, drop and vibration tests, frozen pouch abuse and internal pressure resistance. Significant losses in seal strength were found in four pouches. Separate evaluation eliminated the freezing step as contributing to seal strength reduction. Internal pressure resistance, a MRE requirement, indicated no sample failures. If pouches have sufficient seal strength initially then seal strength reduction appears to be of minor concern.

Introduction

Food and Drug Administration (FDA) approval of packaging for foods to be irradiated has typically been addressed after approvals have been obtained for the food products themselves. In the case of fresh and frozen poultry, commercialization was delayed in part because the typical packaging for wholesale and retail poultry had advanced technically beyond the FDA approvals issued many years ago. Packaging suitable for high-dose irradiated foods was delineated in the 1970's by Natick Soldier Center scientists (*1*). Five laminate systems were developed, investigated and data gathered in anticipation of an FDA petition for high-dose foods. These structures, however, are considered obsolete in the industry where every year new materials and processes create new packaging films. A pouch for a high-dose irradiated food still requires somewhat of a miracle material. The product is vacuum-sealed, frozen to dry ice temperatures to minimize flavor changes, rough handling while frozen, irradiated, thawed, and rough handling again while being shipped or assembled into meals. In addition, even if a pouch employed the film structures outlined historically, separate approvals would be required for different additives or adhesives used to laminate the layers of the material.

Irradiated entrees prepared for NASA have formal FDA approval (March 1995) with a waiver for packaging material. Entrees are irradiated to a minimum dose of 44 kGy. Preparing irradiated entrees for NASA highlighted problems with packaging that was intended for retorting in that a high rate of package failure occurred. Failures typically occurred due to flex cracking at the edge of the vacuum packed product. These failures often were hard to find since the entrees had very little fluid or sauce. One hundred percent inspection was required which involved opening the paperboard carton, inspecting each surface then reinserting into the cartons and resealing the cartons. In order to minimize package failure for NASA production and gather data on a variety of pouches currently available, Natick initiated investigations on five candidate pouches. The pouches included a new Quad pouch developed for the MRE (Polyester/Nylon/Aluminum Foil/Polypropylene), a foreign commercial pouch (Nylon/Aluminum Foil/polyester/linear low density polyethylene), and three generations (#3, #4 and #5) of test pouches that coextruded the inner layers to avoid adhesive use (Figures 1-5). Since the pouches were either commercial products or proprietary research and development pouches, additional information on adhesives was not available. Testing included visual inspections, tensile strength testing, Meade leak tests, drop and vibration tests, frozen pouch abuse testing and burst strength.

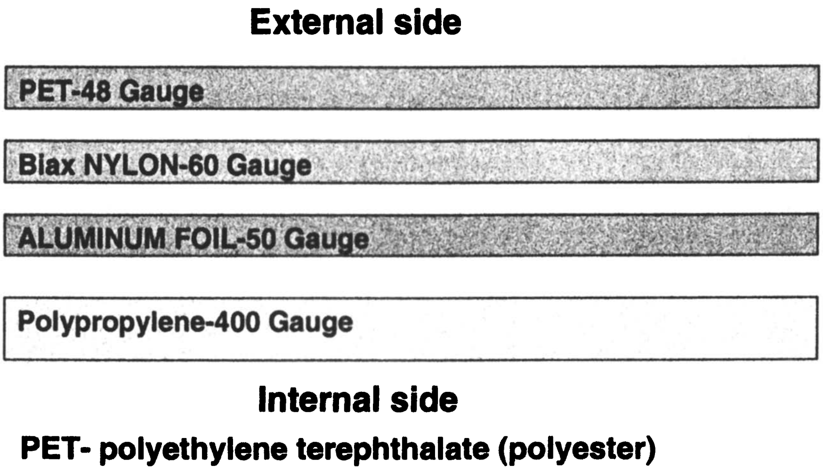

External side
PET-48 Gauge
Biax NYLON-60 Gauge
ALUMINUM FOIL-50 Gauge
Polypropylene-400 Gauge
Internal side
PET- polyethylene terephthalate (polyester)

Figure 1. Quad Retort Laminate.

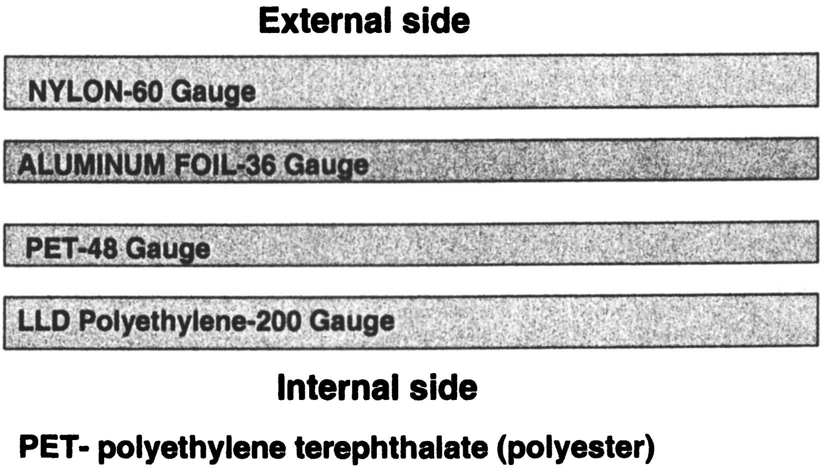

External side
NYLON-60 Gauge
ALUMINUM FOIL-36 Gauge
PET-48 Gauge
LLD Polyethylene-200 Gauge
Internal side
PET- polyethylene terephthalate (polyester)

Figure 2. South African Laminate (General Natick structure circa 1975).

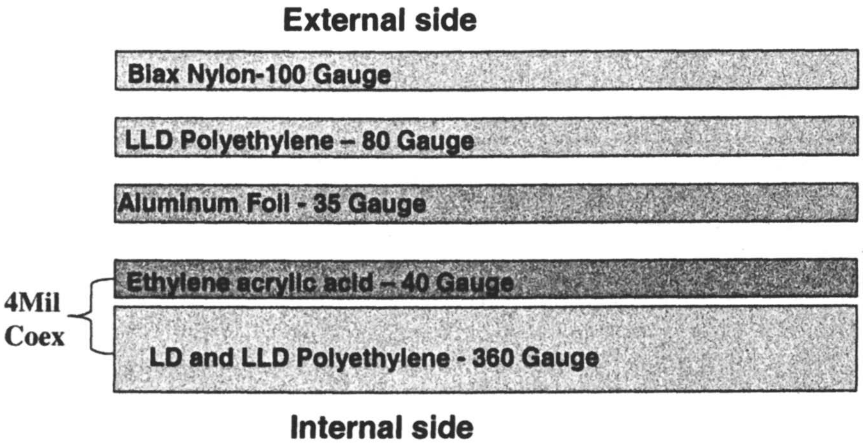

External side
Biax Nylon-100 Gauge
LLD Polyethylene – 80 Gauge
Aluminum Foil - 35 Gauge
4Mil
Coex
Ethylene acrylic acid – 40 Gauge
LD and LLD Polyethylene - 360 Gauge
Internal side

Figure 3. Coextruded Laminates #3.

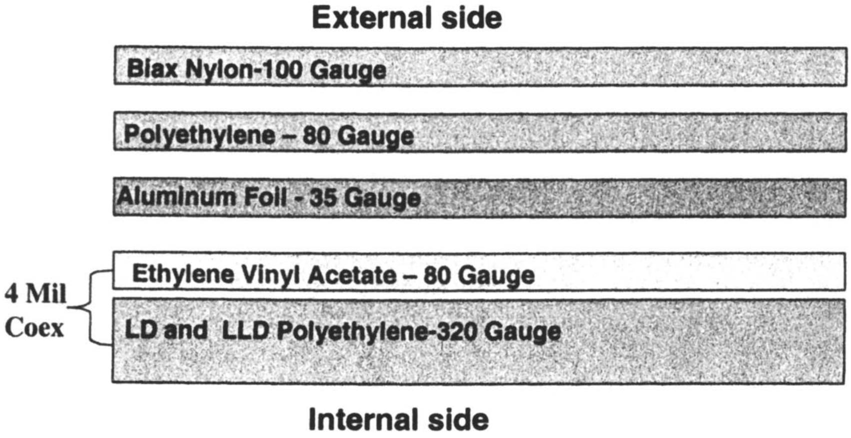
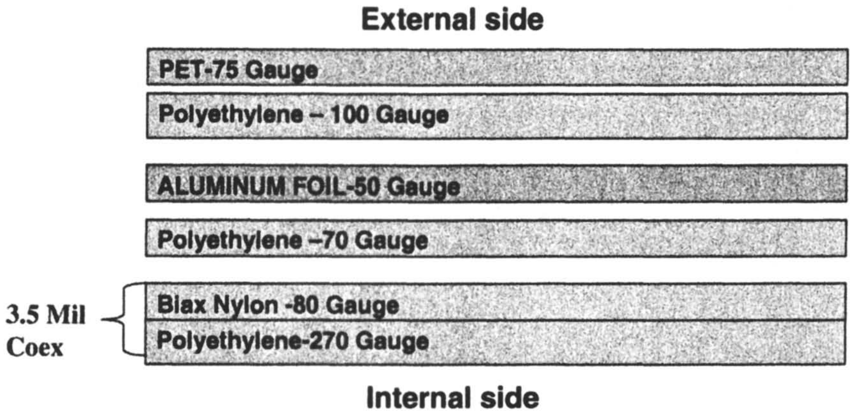

External side
Biax Nylon-100 Gauge
Polyethylene – 80 Gauge
Aluminum Foil - 35 Gauge
4 Mil
Coex
Ethylene Vinyl Acetate – 80 Gauge
LD and LLD Polyethylene-320 Gauge
Internal side

Figure 4. Coextruded Laminates #4.



PET- polyethylene terephthalate (polyester)

Figure 5. Coextruded Laminate #5 (2001).

Evaluations

Twelve samples of pre and post-processed (minimum 44 kGy dose) pouches were inspected for each evaluation. Visual inspection indicated that the MRE Quad pouch had very few defects, the foreign commercial pouch contained surface bubbles (air trapped between laminates) and bottom and closure seal wrinkles. The first two generations of coextruded pouch had grainy surfaces, #3 had side seal delaminations, and #4 had tear notch wrinkles and side seal wrinkles.

Tensile strength testing of the manufacture's seals (ASTM F88-94 (*2*)) indicated that most pouches lost seal strength after processing. The Quad pouch lost 25% (Δ -3.83 lbf) of its original seal strength, the foreign commercial pouch gained 6.3% (Δ +0.73 lbf), the coextruded #3 lost 7% (Δ -0.93 lbf), the coextruded #4 lost 19%(Δ -1.99 lbf), and the #5 lost 28% (Δ -4.17 lbf) in Natick evaluations. The manufacturer of the coextruded pouches conducted alternate tensile strength testing and found a 31% seal strength loss in the #4 pouch after processing. Overall the foreign commercial pouch had the strongest after processing seal strengths (excluding closure seal) with the coextruded #3, coextruded #5, the Quad, and the coextruded #4 following in that order. The general effect of polypropylene tending to lose crosslinks (Quad pouch) and polyethylene (foreign commercial and coextruded samples #3 and #4) gaining crosslinks (*3*) is supported by the results of the seal strength testing and the films' sealant layers. The gaining of seal strength by the foreign commercial and the lower deltas and percent losses of #3 and #4 pouches reflect this. The #5 pouch with a polyethylene sealant layer lost seal strength in similar fashion to the Quad's polypropylene however its initial seal strength was higher than all other samples.

Post irradiation samples of the Quad pouch, the foreign commercial pouch and the coextruded #3 sample were initially leak tested via the Meade test (submersion in water under vacuum at 26 psi). All samples except for one sub lot of the foreign commercial pouch (closure seal only) passed this inspection. The Quad pouch and the foreign commercial pouch were subjected to drop/vibration testing while assembled into typical MRE cases. One case per prototype was evaluated. The Quad pouch was packed in the MRE entrée paperboard carton. The foreign commercial pouch was packed into a padded mailing envelope because of its larger size and our interest in potentially using the package in a field evaluation. The shipping case was subjected to drop and vibration tests in accordance with ASTM standards at room temperature. Following the drop/vibration test the pouches were first visually inspected and then subjected to the Meade test to inspect for leakage. All of the Quad pouches passed while eight of the twelve foreign commercial pouches failed the Meade test at the closure seal.

Testing also included immediate container abuse testing in which pouches were packed in either the paperboard carton (Quad and coextruded pouches) or the padded envelopes (for the foreign commercial pouch). This test consists of a drop down a slide at a height determined by the weight of the product (Figure 6).

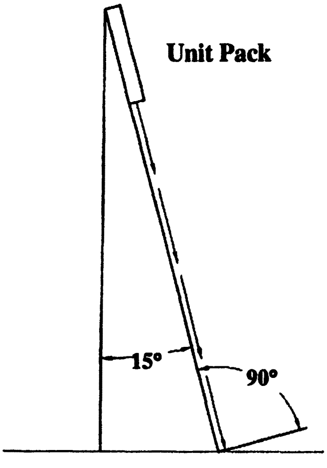


Figure 6. Immediate Container Abuse Test Apparatus

Pouches were dropped at room temperature, examined visually then frozen to -16°F and dropped, visually examined and then Meade tested. All samples of the Quad pouch passed, four of twelve foreign commercial pouches failed the final Meade test. Fewer samples of the coextruded pouches were evaluated, one of five #3 pouches failed the Meade test and both of the #4 pouches tested failed the Meade test after immediate container abuse.

A burst strength test used for evaluating the MRE retort pouch seals was also employed to evaluate the post-processed pouches. The test consists of subjecting the pouch to 20 psig of internal pressure held constant for 30 seconds. All samples passed this MRE retort pouch requirement.

Seal Strength-Process Effect Breakdown

It was unclear what step in the processing contributes to the loss of seal strength. The Quad pouch was selected to further investigate the loss of seal strength since that was the pouch chosen to package product processed under the overall program. Evaluations were conducted on samples drawn sequentially from the lot of pouch material used for all previous evaluations. First, 24 units of previously processed turkey slices were vacuum packaged and alternate samples were frozen to -20°F for a minimum of 48 hours prior to testing. No significant difference in bottom seal tensile strengths were found and a significant increase in tensile seal strengths were noted in the side seals after freezing (Table I).

Table I. High Dose Packaging Tensile Strength Testing-Quad Pouch Load at Peak (lbf)

	Total Process		*Freezing*		*Irradiation*	
	Pre	Post	Not Frozen	Frozen	Pre	Post
Side Seal	14.49	10.66*	13.39	13.73*	12.51	12.50
Bottom Seal	14.04	11.03*	11.50	11.75	13.66	10.86*

* Significant ($P<0.01$)

A second evaluation was conducted on sequential/alternate Quad pouches without product where half the pouches were irradiated to a minimum 44 kGy dose. No significant differences in side seals were found but a significant drop in bottom seal strengths were noted. All tensile strength values were well above values that would be of concern (less than 6.0 lbf) but there are no established lower limits for tensile strengths.

The evaluations indicate that the freezing step is not a contributor to the loss of tensile strength. The irradiation step significantly contributes to seal strength loss. The total process, which includes rough handling (shipping) in the frozen state, may also contribute to seal strength loss. If the pouch has sufficient initial seal strengths, the total process seal strength loss is not of major concern since the entire process does not jeopardize the total package integrity.

Future High-dose Packaging

Additional packaging efforts include development of a generic film through the International Research Coordination Meetings (RCM) for High-Dose Processing. A generic laminate that would use a polyester film as a sealant layer has been proposed (Figure 7).

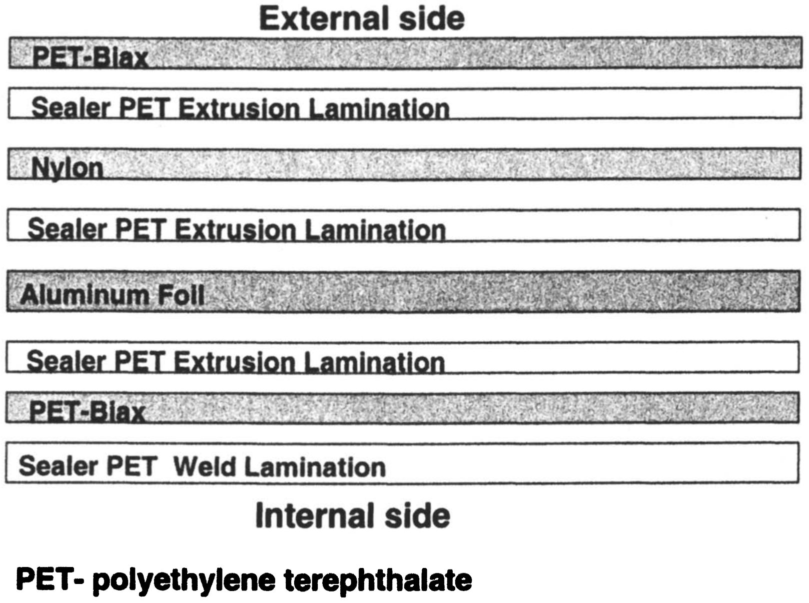

External side
PET-Biax
Sealer PET Extrusion Lamination
Nylon
Sealer PET Extrusion Lamination
Aluminum Foil
Sealer PET Extrusion Lamination
PET-Biax
Sealer PET Weld Lamination
Internal side
PET- polyethylene terephthalate

Figure 7. Proposed Laminate for Irradiated Foods

Elimination of adhesives with the use of a polyester film would simplify an FDA petition for packaging of high dose foods. Also of interest for high-dose packaging is a Natick Science and Technology program investigating nanocomposite films which would not only eliminate adhesives but the laminated layers themselves in an entirely new type of film. Until then the Meal, Ready–

to-Eat Quad retort pouch is being used for NASA production because of availability and it has proven a successful package. Designing and producing high-dose irradiation packaging will probably not be as problematic as obtaining the regulatory approvals.

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October 23, 2009 | http://pubs.acs.org
Publication Date: January 2, 2004 | doi: 10.1021/bk-2004-0875.ch020

Chapter 20

Irradiation of Mail: Effects on Archival Museum Materials

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One aspect of the response to a biological attack using anthrax spores sent through the mail has been to use electron irradiation to treat the mail. This irradiation procedure has altered the materials and objects in ways that were probably not anticipated. The mail was damaged by both thermal and radiation processes. Business, government, and personal records are all sent through the mail and damage to these records must be assessed for archival reasons. The irradiated paper became discolored and lost its ductility, i.e. became brittle, and components of some ballpoint pen inks were chemically altered. Thermal effects caused the sticking together of pages in books and journals. Photographic images, slides, and negatives were damaged and often destroyed. Objects such as computer disks were also damaged by melting or softening of their plastic components.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

Introduction

The end of 2001 was a period that produced major changes in the security procedures of the United States. The finding of anthrax in letters sent as a terror weapon changed the way the mail was processed. In order to insure the safety of the mail the Post Office decided to use an irradiation procedure to kill any biological warfare agents such as anthrax spores. Initially, this procedure was applied to mail arriving at the mail processing facility serving the Capitol area, with the possibility of expanding to the treatment of all mail. This procedure had unintended consequences.

While electron beam radiation has been used successfully for some time in sterilizing food, the high doses chosen in order to kill virtually all anthrax spores, 50-100 kGy, presented new problems not encountered at the lower doses used for food, 7-20 kGy (*1,2*). Not long after the start of the irradiation of mail, problems became apparent. Letters were found to be yellowed and often damaged and embrittled. Objects such as photographic materials were damaged. There were reports in the press of gems changing color and stamp and coin collectors became apprehensive after anecdotal reports of damage (*3*). Objects mailed to museums and archives in the area, including those of the Smithsonian, also were damaged.

The damage to objects could be separated into two categories, that due to the irradiation itself and that due to the thermal effects associated with the process. Radiation doses were higher than normally used because of the need for certainty in combating the threat. The high temperatures reached in the process were most likely due to the mechanical aspects of processing. Radiation doses were expected to be about 50-100 kGy depending upon the number of passes and heating was thought to be a function of thickness of the irradiated bulk packages.

Modern society generates an ever-increasing amount of documentation. People, government and business require that this documentation be preserved for a minimum amount of time. Because of the problems associated with some irradiated mail, archival materials were often copied, resulting in an increase of work and affecting storage space. Since the effects of the changes in mail processing seem to have altered the lifespan of mailed objects, our laboratory investigated some of the effects upon materials used to document the activities of our institution.

Experimental

A large number of postal specimens were available for study (any mail sent to our central Washington DC mailing address). These included paper and photographic materials as well as plastic holders and containers. The electron irradiation procedure used by the post office consisted of combining mail in bundles about 5 inches thick, sealing the bundles in plastic bags, and irradiating them at 10 MeV for a minimum dose of 50 kGy per pass, with each bundle given 2 passes (*2*). Other test specimens irradiated separately specifically for this study were treated at the Florida Accelerator Services and Technology (F.A.S.T.) Facility, Gainesville, Florida, at 5.2 MeV at 250 microamps for a total dose of approximately 260 kGy. This was anticipated to be 2 - 5 times the dose at postal facilities and although at a lower dose rate, it was expected to give indications of the problems to be encountered.

Tensile tests on paper specimens were performed on screw driven tensile testers as described previously (*4*). Specimens were cut to approximately 125 mm x 6 mm x 0.15 mm and equilibrated to 30% RH and then run at ambient temperature and 45% RH. Tests were performed on standard envelopes and paper used in the course of business (both irradiated and unirradiated samples) and on irradiated samples of Whatman paper that had been characterized previously.

Ink samples were applied to Whatman papers and allowed to dry for several weeks to minimize the effects of evaporation of ink solvents. Samples were tested by thin layer chromatography (TLC) on silica gel plates using two different solvent systems as described previously (*5*).

L*a*b* values and changes in color were measured and calculated on a HunterLab Ultrascan spectrophotometer. Most color changes were apparent to the unaided eye.

Paper and plastics were heated in dry ovens at temperatures expected to be encountered in the process of irradiation of mail. By measurement or deduction, the temperatures reached by the specimens during irradiation were determined to be between 80 and 130°C and possibly higher. Ignition of paper and other materials had been reported, implying quite high temperatures. In addition, certain paper specimens were heated in ovens with controlled RH since this has been found to produce aging processes similar to those that occur during natural aging (*6*).

Moisture uptake experiments were performed in chambers with controlled RH environments and weight measurements were made using a Mettler AT201 balance accurate to 0.01 mg.

Determinations of glucose and xylose concentrations were performed on some specimens. A sample of paper (about one gram) was extracted with stirring in 25 ml of deionized water for at least 2 hours. The extract was filtered, separated into 5 ml aliquots, and then evaporated under vacuum. The residue was derivatized as follows: 0.1 ml STOX, a commercial reagent containing hydroxylamine hydrochloride and O-phenyl-β-D-glucopyranoside (internal standard) in pyridine, is added to the residue of one of the aliquots and heated at 70°C for 1 hour to convert carbonyl groups and any cyclic hemiacetals to the oximes. 0.1 ml hexamethyldisilazane and a drop of trifluoroacetic acid is added. The *per*-trimethylsilylated supernatant is analyzed by gas chromatography on a DB-17HT column with flame ionization detection. The chromatograph is programmed to start at 50°C and then rise to 330°C at a rate of 10°C per minute.

Sugars were identified by comparison of their retention times with standards as well as by GC-MS. The procedures have been described previously (*6*).

Results

Polystyrene

Polystyrene is a component of many postal envelopes and was found to be a good indicator of damage. The "windows" of business envelopes were often deformed and in some cases contracted to small brittle fragments. The contractions were due to the release of "casting" strains within the windows. Experiments with similar materials showed that there was little damage at temperatures below 100°C even for an extended period of time, e.g. 30 minutes. Where these fragments were in contact with printing they often reproduced in miniature the printing beneath. Computer disks of polystyrene sent through the mail were found to be deformed in some cases. Simulations that involved heating disks, windows, photographic mounts, etc., showed that temperatures of at least 80 to 110°C were needed to replicate the damage seen in many postal specimens. Damage in postal specimens was not universal, however, and probably depended on their location in the irradiated bundles. Reducing the thickness of the irradiated packages will minimize damage due to thermal effects (2).

Spunbonded Olefin

Envelopes made from spunbonded olefin were also received in conditions indicative of high temperature. These materials will permanently deform at temperatures above 107°C and will melt at 135°C (*7*). Journals mailed in packages made of these materials were stuck to the envelope, probably due to a combination of effects from both changes in the inks present on the journal cover and the softening of the envelope itself. Heated envelopes exhibited a softening range of 118 - 130° C.

Photographic Emulsions

Photographic emulsions were found to be at risk given the high temperatures reached during the irradiation process. Cracking and separation of the emulsion was noted on transparencies and in some cases blackening and powdering as well. Polystyrene slide mounts were found to be deformed in many cases. Tests on similar materials indicated that the temperatures reached were over 100°C.

Inks and Toners

Ballpoint pen inks were analyzed after irradiation and found to be relatively stable in color. One surprising finding was that on TLC analysis some of the blue pen inks had a new more rapidly moving component similar in color to the bulk color of the ink. While not of immediate practical concern, this may have some forensic implications.

Printing inks and photocopy toners in particular were found to adhere to adjacent pages of photocopies, journals and magazines. This is probably due primarily to softening of the resin binders at temperatures similar to those that affect polystyrene. However, wrinkling and tide lines indicative of the effects of liquid water also were observed. Liquid water probably resulted when water driven out of the hottest regions of the bundles condensed in cooler areas. The combination of softened ink binders and moisture condensation lead to severe "blocking" or sticking problems. This has become a serious problem at the Smithsonian Institution Libraries and elsewhere.

Paper

The most obvious effect on paper is the change in color. The change is a distinct yellowing and muting of color which is slight immediately after irradiation but which intensifies with time. Color measurements, L*a*b*, for a typical paper six weeks after irradiation showed a general distinct darkening ($\Delta L^* = -1.13$) and a color shift to the yellow ($\Delta b^* = 4.68$). There was a small change in a*, ($\Delta = -0.29$). Yellowing occurred whether or not the paper had any lignin content. Mail that had been irradiated could be identified almost universally when compared to unirradiated mail.

Significant physical changes to irradiated paper, particularly in the mechanical properties, could be determined. Whether these changes were due to thermal effects, radiation, or a combination of both were investigated.

Samples of Whatman #1 MM paper were sent through the mail or were irradiated elsewhere. Whatman #1 MM paper is a rugged paper, which is primarily a β-linked glucose polymer (α-cellulose) with no hemicellulose or lignin. Even at a high dose of 250+ kGy the Whatman paper remained strong and somewhat ductile although measurements showed some loss of ductility. A tensile test of the irradiated paper versus an unirradiated paper is shown in Figure 1. As the strain-at-break decreases to less than 0.01 (i.e. 1%) the ability of the paper to be folded, deformed, or manipulated becomes increasingly less. The irradiated paper becomes more susceptible to damage.

The changes in the mechanical properties of business paper sent through the mail were even more drastic. These papers, which are less rugged than the Whatman paper, have often been found to be damaged and torn when received. If the loss of ductility becomes severe then the movement of the business paper through the postal machinery and other handling could lead to the observed rips

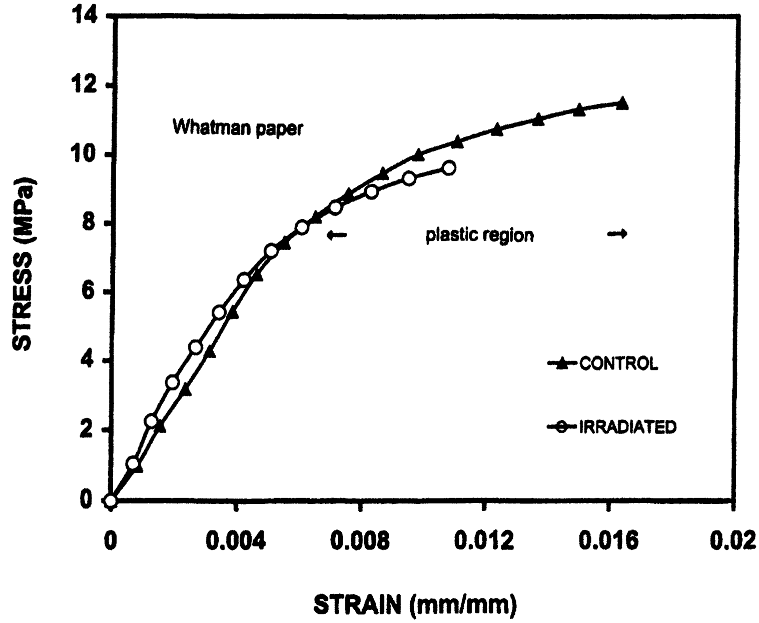

Figure 1. The stress-strain plot for two samples of Whatman paper in the same direction. One specimen was irradiated to a total dose of 257 kGy. There is a loss of ductility which if continued increases the possibility of damage on handling.

and tears. Figure 2 shows the stress-strain plot illustrating the loss in ductility of a business envelope sent through the postal system as compared to an unirradiated envelope. It can be seen that the paper has lost almost all of its plasticity, the ability to tolerate large strains by permanently deforming. It does retain its elasticity as is noted in the initial linear portion of the stress-strain curve. At these lower strain values the paper can be deformed without permanent change. Even though this paper was initially quite tolerant of large strains, it has now reached a point where it is susceptible to damage through handling or folding. This does represent an extreme in damage and is not representative of all mail that is irradiated. It is meant to demonstrate the potential for damage under the most adverse conditions.

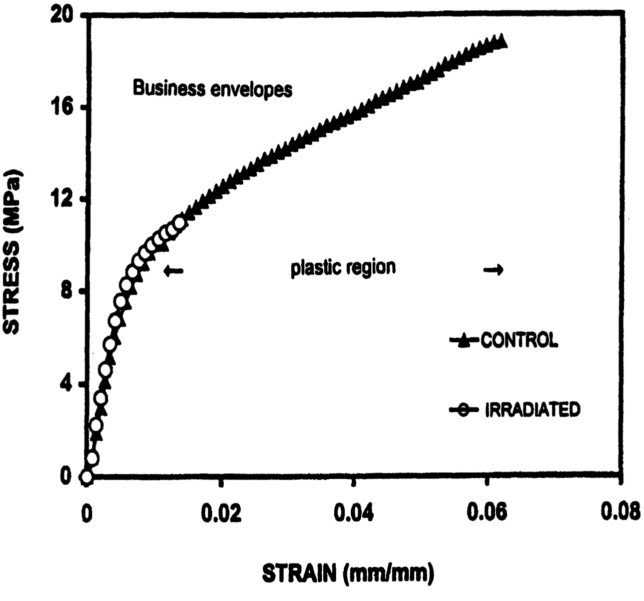

Figure 2. The stress strain plot for two business envelopes, one irradiated when sent through the mail and the other a control. The loss of ductility in the irradiated envelope is severe and represents an extreme of damage to mailed objects.

There are also significant changes that occur chemically. These changes are different from those seen in the control papers, or papers that have undergone natural or accelerated aging.

The chemical consequences of the irradiation of paper may be seen in the comparison of degradation products as illustrated in the gas chromatogram in Figure 3. Differences in the profiles of extracts of the control and irradiated specimens are apparent. Aqueous extracts of irradiated papers showed a significant increase in the amount of extractable, soluble material. This soluble material represents degradation products since unirradiated paper has only small amounts of soluble components. The degradation product distribution of the irradiated samples is very different from the distribution one finds on examining naturally aged papers.

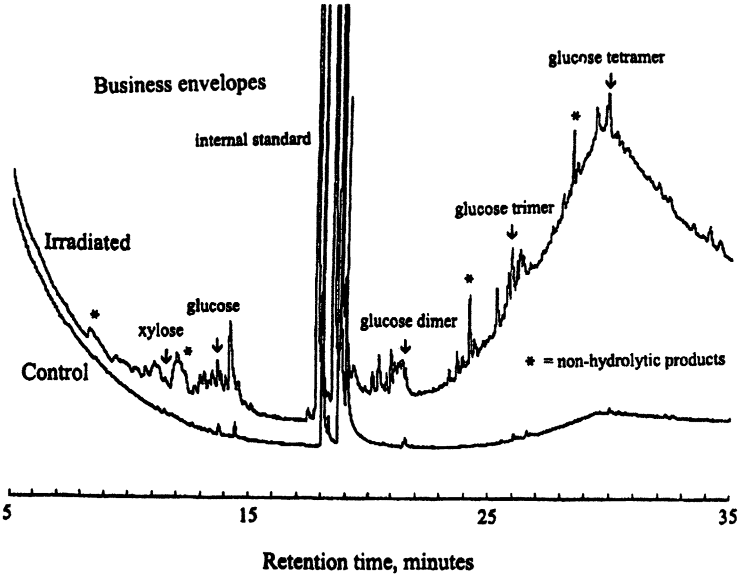

Figure 3. Comparison of the gas chromatograms of the per-trimethylsilylated oxime derivatives of extracts of unirradiated and irradiated paper sent through the mail. Typical amounts of the products of natural aging are seen in the unirradiated sample while increased amounts of xylose, glucose and glucose oligomers as well as other compounds are seen in the irradiated sample.

The primary degradation process during natural aging is hydrolysis, and the typical soluble products found on analysis of naturally aged papers include xylose, glucose and a series of glucose oligomers resulting from hydrolysis at different points along the cellulose chain. Hydrolysis of the hemicellulose found in papers containing wood pulp also yields xylose and some glucose as well. Smaller amounts of other compounds including products of oxidation and chain scission are also present, but in smaller amounts. The control paper has only minor amounts of these compounds as would be expected in a "new" sample. The irradiated samples show increased amounts of hydrolysis products, but these are overshadowed by the production of other compounds from other reaction paths. One may postulate that the hydrolysis products were formed at high temperatures before the original ambient water was driven out by heating. "Tide lines" found on some mailed items showed that there was water present beyond that normally contained in the paper. The condensation of water in some regions of the packets is due to uneven heating of the bundles, which drives moisture to the cooler regions. The other products are formed either through scission or free radical reactions directly caused by irradiation, or by thermal scission or oxidation resulting from the greatly increased temperatures.

To determine changes in moisture uptake at high relative humidity, thermally treated and irradiated papers were compared with paper sent through the mail. Paper heated to 105°C in a dry oven (less than 2% RH) crosslinks and loses its ability to take up moisture at high RH, e.g. 100% RH. Whatman paper previously heated at 105°C for 1 hour takes up 10% less water than unheated paper. The same paper irradiated to approximately 260 kGy dose by an electron beam took up 51% less water. Whatman paper mailed to our facility through Washington DC showed a reduction in the ability to take up water of 43%. Since it is doubtful that high temperatures would be sustained for over one hour during the irradiation process, this change in the paper would be primarily due to the electron beam and induced free radical reactions.

Discussion

The treatment of mail by electron beam irradiation can create damage by both thermal and radiation effects. In the procedures in use at the time of this work, small mailed items were packaged in bundles up to five inches thick sealed in plastic bags, and then irradiated usually with two passes to insure a minimum dose of 50 kGy throughout the bulk sample. The overall effects observed by the postal patron are due to high temperatures, electron beam irradiation, and some moisture effects.

Mailed paper was found to have yellowed and embrittled, and address windows either deformed or contracted. Printed materials may be stuck together either from softening and melting of ink binders or from the movement of moisture within the irradiated package. Plastic materials such as slide mounts or computer disks may be seriously deformed.

Because the heating of the packages of mail causes moisture to migrate, water can be driven from the hottest portion of the block of mail and condense in the cooler regions, especially on the inner surface of the plastic bag. The resulting movement of water can cause blocking or adhesion of paper, the running of inks and dyes, and the formation of tide lines. In conjunction with the softening of resins in printed materials this can lead to inseparable masses of paper, particularly in the case of journals.

Changes in the thickness of the irradiated packages and lower total doses will lower the temperature and reduce the possibility of thermal damage and associated moisture damage.

Pending the adoption of methods that can reduce both the biological hazard and damage to mailed items, items that cannot or should not be irradiated are shipped by other means. Archival materials such as paper are being duplicated where practical.

Acknowledgments

The authors would like to thank Walter Hopwood and Caroline Solazzo for technical assistance and Dr. Burrell J. Smittle, Division of Plant Industry of the Florida Department of Agriculture and Consumer Services, Gainesville, Florida for irradiation of some samples.

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Chapter 21

Outlook for Food Irradiation in the 21st Century

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> " *I don't pretend to have all the answers. But the questions are certainly worth thinking about.*"
>
> Arthur C. Clarke

Introduction

As we move forward into a new century and a new millennium it is appropriate to reflect a little on what the future holds for food irradiation. Will it finally attain a major presence in the food systems of the world, or will it continue in its previous ambivalent state, chronically offering mainly promises of imminent fruition? While it is difficult, perhaps even foolish to attempt, to forecast the future of this area of endeavor, there is much reason for optimism about what lies ahead. A variety of indicators from a broad cross-section of the food industry and indeed of society at large suggest that the present time is truly a watershed point along the road leading toward a significant commercial and industrial reality for this technology. After decades of optimism alternating with disappointment and frustration, all driven by passionate promotion and equally passionate opposition, it appears that a new stage in the evolving status of food irradiation is crystallizing before our eyes. This does not yet guarantee unfettered progress, but it has allowed it into the crucible of the market place, which is the ultimate arbiter of real success for any offering of new technology. The market place will decide what the future holds.

In Irradiation of Food and Packaging; Komolprasert, V., el al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

The Future will be very Different from the Past

" I tell you the past is a bucket of ashes." Carl Sandberg

Recent progress in the USA displays the characteristics of a 'breakthrough' advance in the implementation of food irradiation. For example, in the past the support for irradiation came mainly from researchers in university and government laboratories, and a few public health agencies, along with members of the radiation industry fraternity. The food industry, from farm through manufacturers and retailers, kept their distance and effectively stymied any possibility of significant implementation by the simple expedient of ignoring it and thereby dooming it to languish with nowhere to go. In stark contrast with this is the present climate where the food industry and other stakeholders have done an about face and have now begun to enthusiastically and proactively engage in its implementation. Thus the current 'facts on the ground' are very different from the previous *status quo*, and the future promises to be qualitatively very different from the past concerning behavior of irradiation in the market place. For this reason one cannot use the statistics of past performance as a basis for predicting the behavior in the future. In this essay an attempt is made to discern the outlines of the future of this technology by examining the fundamentals underpinning its *raison d'etre,* and how these are interacting with the overall consumer and social environment. It is these fundamentals that ultimately determine the true worth of any proffered technological advance.

Overview of the Food Irradiation Landscape – Where it is Today in the USA and Elsewhere

In the USA food irradiation is currently (2003) enjoying an unprecedented and rapidly growing level of acceptance across a broad cross-section of society (*1, 2*). This includes food processors, industry associations, retailers, foodservice operators, regulators, public health officials, academics, politicians, consumers and, very important, investors. Across the country consumers can now choose to purchase irradiated products, primarily ground beef but including growing amounts of poultry and tropical fruits, in thousands of retail outlets. This is in marked contrast to the situation only two or so years ago, when the number of retail outlets carrying irradiated product could be counted on the fingers of one hand. Almost on a weekly basis significant numbers of additional retail outlets are joining the ranks of those already offering the choice of irradiated products. The list of retailers involved includes many of the best known names in the retail grocery sector. On the production side processing capacity is increasing, with new irradiation facilities being built in several places across the country to serve the needs of a growing number of food processors who are adopting the technology. This expansion is supported by investments in several companies, both public and private, vying to become players in what is seen as a developing opportunity.

Trade in irradiated tropical fruits and vegetables is also displaying significant activity, with Hawaiian produce already entering into mainland USA, and additional streams of products originating in Brazil and elsewhere poised to gain entry into that market. These trade developments are the direct result of the recent establishment of regulations by USDA-APHIS (*3*) allowing the use of irradiation as a quarantine security treatment for certain common pests associated with agricultural products. There is significant interest in several producing countries to take advantage of these developments as a means of gaining access to new markets for their agricultural production. Indications are that rapid growth in this use sector can be expected in the near term future.

In a number of countries around the world, for example China, France, Mexico, South Africa, Thailand and others, food irradiation is already being used in a variety of specialty applications such as spices, seafood and various ingredients, among others (*4*). These applications have been on-going at a more or less stable level for a number of years. They represent basically niche markets, sharply delineated along both geographical and product lines, and with only limited visibility in the marketplace. The current activity in the USA differs from these on-going special applications because it represents the leading edge of a widespread adoption of irradiation for mainstream consumers and products, and is growing rapidly. In that regard the USA is the stage on which the struggle to meaningfully implement this technology is playing out before the world.

Although progress in the past couple of years has been most impressive, as outlined above, yet today even in the USA only a tiny fraction of candidate products are being processed by irradiation. The bulk of the opportunity still lies ahead, available for capture by those with the vision and resolve to do so.

The Forces Driving Implementation

There are two major fundamental needs that can be addressed by irradiation and which are serving to drive implementation forward in the food industry. First, are needs in the realm of food safety? Failure to have these needs met is directly manifested in outbreaks of food-borne illness and recalls of contaminated product. In particular an effective means for elimination of *E. coli* O157:H7 in ground meat products and of *Listeria monocytogenes* in ready-to-eat foods is required, since both of these food-borne pathogens are in the regulatory category of “zero tolerance” (*5, 6*). Outbreaks and recalls are associated with much pain and suffering of those directly afflicted, and with considerable financial penalty in the form of remediation costs, lost business, litigation, and loss of reputation to the manufacturer of the contaminated product. In some instances manufacturers, even some very large ones, have been forced out of business by the fallout of a major recall (*7*). Consumers in the USA have become acutely sensitized to food safety issues, while food processors and retailers have become acutely aware of the risks and liabilities flowing from instances of contaminated food products. For meat industry executives, irradiation represents a powerful new tool in their risk management toolkit. Further, in addition to the concerns about financial liabilities associated with food safety issues, there are moral and ethical issues involved, and there is a growing acknowledgement among industry leaders that adoption of irradiation is 'the right thing to do'. It is just 'not right' to be putting into commerce food products which are not as safe as the best technologies currently available, and which are cost effective, can make them. Today both the consuming public and the food industry are increasingly aware of the extra protection offered by irradiation against food safety hazards and are increasingly seeking this benefit.

A second major need is in the realm of quarantine security for agricultural products in national, international and inter-regional trade. Quarantine security objectives require that a system be in place to protect the ecology and agriculture of the importing region from pests that may be present on imported goods, while at the same time facilitating trade between regions (*8*). Such a dual purpose quarantine system is a critical essential element in the on-going expansion of global trade in agricultural products. In turn, such expansion in trade opportunities is critical to the economic growth of many developing nations. Of the technologies available to fill this quarantine need, irradiation is increasingly recognized as an effective, versatile, convenient and economical solution. The flow of irradiated agricultural products in trade has already begun, led by importation of Hawaiian tropical fruits into mainland USA (*9*). While the volumes of product from Hawaii are still relatively modest at this time, the real significance of this development is that it establishes the validity and practicality of irradiation in this application. Already there are several new initiatives to implement irradiation to meet the quarantine security requirements for agricultural products from other regions, most notably Mexico, Brazil, Thailand and the Philippines. The opportunity for future growth involving trading partners around the world is significant for both the agriculture and irradiation industries.

There are additional benefits flowing from radiation treatment of food (*10*), such as shelf-life extension or functional modification, and in some cases these offer additional incentive for adoption of the process. However, as drivers of the primary expansion of irradiation these are of secondary importance, at least in the USA.

The Path that has taken us to the Present Reality

There is a string of specific events that together are largely responsible for the favorable change in attitude towards irradiation. The triggering event is widely acknowledged as being the outbreak of food-borne illness in northwestern USA in 1993, when beef burgers contaminated with *E. coli* O157:H7 were sold through a popular fast food restaurant and caused several hundred illnesses and four deaths (*11*). This unfortunate event shook up both the food industry and consumers and served as a wake-up call that placed food safety at the top of the priority list for all those involved with the production, selling and consumption of food. Prompted by the public outcry following this outbreak the food safety regulations in the USA were extensively revised for the first time in almost a century, leading to promulgation of the aptly named 'mega-reg' by USDA-FSIS in 1996 (*12*). The new regulatory landscape made it mandatory for all meat and poultry manufacturing plants to develop and implement a comprehensive HACCP program to deal with food safety issues for each of their products. (Seafood and fruit and vegetable juice plants also require HACCP, but these foods are not yet approved for irradiation.) Of course, to be effective HACCP plans require scientifically validated critical control points (CCPs) to reduce or eliminate the specific identified hazards. For microbial hazards in raw meat and poultry, and especially for minced (non-intact) products, irradiation is well recognized as being an excellent CCP (*13*). There really aren't any effective practical alternatives to irradiation for eliminating microbial pathogens from raw ground meat products. The fact that irradiation can be applied as a terminal treatment, after the product has been packaged and sealed, makes it especially suitable for this use.

In 1997, after HACCP had already been implemented in the largest plants, the meat industry was rocked by a massive recall at one of the major meat processors in the USA, involving ground beef contaminated with *E. coli* O157:H7. This was the largest recall in history up to that time, and resulted in the company bring forced out of business. Since that time there have been several additional massive recalls (*14*), each affecting many millions of pounds of product, involving both ground beef contaminated with *E. coli* O157:H7, and further processed ready-to-eat foods like hotdogs and deli meats, contaminated with *Listeria monocytogenes*. In total, scores of people were sickened in these incidents and there were a significant number of deaths. In addition to these massive recalls there have been many, many smaller ones, ranging in size from tiny (hundreds of pounds) to quite large (hundreds of thousands of pounds). These recalls continue as a quasi-random series of events, with two of the most recent massive ones occurring late in 2002. There is little reason to expect that this situation will self-correct. Thus it appears that in spite of the current best efforts of the food industry, instances of contaminated food stubbornly persist.

Costs precipitated by food-borne illness and recall events can severely damage the financial health of the companies involved. Besides the direct costs of recalling the product, effecting remediation in the plant, covering the loss of business revenue, and repairing the damage to the company's reputation, there is loss of investor confidence. This impacts on share prices and in some instances has severely eroded the value of shareholders' equity in the company. Further, injured parties involved in an outbreak of food-borne illness are increasingly launching lawsuits against the responsible companies to receive financial compensation for the harm that was visited upon them (*15*). Currently there are a number of such lawsuits before the courts, including some class action ones. Such settlements can each be in the millions of dollars.

Awareness of the staggering financial penalties associated with food-borne illness events caused by a company's contaminated product has turned food contamination into a high profile risk management issue for company executives. More and more food industry managers are recognizing that instances of contaminated food can strike even the best run companies. Increasingly irradiation is viewed as a form of insurance policy protecting against the human and financial disasters that can flow from these unfortunate events.

Together these several factors and considerations have placed irradiation in a new, much more favorable light. The implementation progress that is so evident attests to the persuasiveness of these factors and conditions. In corporations as in individuals, the instinct for self-preservation runs deep.

Why Now?

It is instructive to examine why these changes are happening at this time. Is it due to an unusual, transient convergence of essentially random factors creating the present favorable conditions for progress? If so, then perhaps this present set of circumstances will soon dissolve and the pressure for implementation will subside. Alternatively, it may be that there has been some fundamental change in the underlying social condition such that a new era is upon us. One possibility is that the true incidence of contaminated food and of food-borne illness actually has increased in the last decade, but that seems unlikely, since the incidence of contamination with *Salmonella* spp. and *E. coli* O157:H7 in products has actually been dropping somewhat since the implementation of HACCP (*16*). Rather, it seems more likely that the increasing number of food-borne illnesses detected and recalls issued reflects advances in the ability of public health scientists to detect even widely disseminated outbreaks of disease stemming from a common cause, and to trace the responsible contaminated product back to the manufacturer. In particular, the establishment of the Foodnet and the Pulsenet initiatives (*17, 18*), involving the Centers for Disease Control and Prevention (CDC) along with public health authorities in several co-operating states, enables a ready comparison of the genetic fingerprints of microbial pathogens isolated from individual cases of gastrointestinal illness. This information allows identification of any cases arising from a common cause, and ultimately to trace it to a particular contaminated food. Previously any common-cause outbreaks of food-borne illness comprised of disseminated cases in a background of unrelated similar illnesses could not readily be detected. Those days are over.

In future of course the ability to detect such outbreaks of food-borne illness will undoubtedly be even further improved. Thus, the era when contaminated food might enter the distribution system, be consumed and cause illnesses without being detected is drawing to a close in the USA. As similar detection capabilities are expanded into new regions and countries it is likely that the story as already experienced in the USA will be repeated, expanding the prospects for food irradiation as an important part of the solution to this problem.

Our Modern Food System is a Double Edged Sword

Over the past several decades, especially since the Second World War, there have been dramatic changes in the way in which our society is organized to provide the necessities of life to its members. There has been a pronounced movement away from the previous predominantly rural way of life based on agriculture, with an overwhelming proportion of people now living in cities. The evidence for this is readily witnessed on any casual drive through rural America. Such *urbanization* has had a profound effect on the way in which people make their living and feed their families. In the USA today a mere 2 or 3 percent of the total population are engaged in primary production on farms (*19*) and are producing an abundance of food that easily feeds their more numerous city cousins, with a surplus remaining for export. In real terms food is cheaper now than it has ever been. It is truly remarkable that the average American spends only about 10 percent of his or her income for food, and is able to choose a more varied and better diet than ever before. This abundance is made possible by a modern food production system that is very different from that which has fed mankind in the past, when the monotony of a very limited diet and the endless hard work of producing food was interrupted only by the threat of famine.

Around the world, all modern food systems are characterized by the attributes of mass production, central processing and widespread distribution, intertwined with a high level of advanced technology. These attributes underlie the high efficiency that allows production of the abundance of food evidenced everyday in any supermarket across the country. Modern cities could not exist if our modern food system did not exist. Unfortunately there is an Achilles heel in the system, namely that those same attributes which make the food system so efficient also make it vulnerable to the spread of food-borne microorganisms and pests. When production units were small and essentially isolated from each other any contaminated food would affect only the local population, often just a single family unit. One can further speculate that such isolated or semi-isolated production units represented fairly closed ecosystems, so that the dwellers in each would build up immunity to the local strains of pathogens. This allowed people and pathogens to co-exist in at least a quasi-equilibrium. In contrast, in today's circumstance when something goes wrong and contaminated product is produced and distributed over large geographical regions, there is potential for infecting large numbers of people who have never encountered the particular strain of pathogen that might be involved. The food you consume today may have been produced almost anywhere, and any contaminating microorganisms are likely to be unfamiliar to your immune system. Thus the food industry must have safeguards in place to prevent its products from serving as a vehicle for dissemination of pathogens, as well as pests. However there is a caveat, which is that *while the necessary safeguards must effectively prevent spread of food-borne agents that can harm either the consumer or the environment, they must not interfere with the high efficiency which is required of the system.* Fortunately the food industry has developed many excellent safeguards and by and large the food we eat today is safer than it has ever been. Nevertheless, as recent experience has shown, outbreaks of food-borne illness are still with us, people do get sick and die of such illnesses, and so obviously there is need for further improvement (*20*). Irradiation represents only the latest weapon in the continuing series of safeguards addressing that need, but it is a particularly powerful one.

We Can't Go Back to Simpler Days

The changes stemming from urbanization are irreversible. It is inconceivable that we could (or would want to) go back to the simpler days of previous generations. Thus, along with our urban lifestyle the modern food system is here to stay, including also the problems inherent in it. In so far as irradiation can contribute to reduce or eliminate these problems, the need for irradiation will not go away.

Challenges to Growth

Continued expansion in the use of food irradiation faces serious challenges. One of the most obvious is the need to loosen the regulatory strictures on its expanded use. In principle this should be relatively straightforward, since *Codex Alimentarius* has endorsed irradiation as a food process for all foods regardless of absorbed dose as long as the purpose of irradiation is technically legitimate, and a *Codex* standard for food irradiation along with a recommended *Code of Practice* is in place (*21*). Unfortunately in practice most countries have ignored the *Codex* recommendation and continue to grant clearances in response to petitions on an item-by-item basis as if irradiation were a food additive. This is a slow and expensive process and constitutes a severe bottleneck in the attempts to apply irradiation to many foods that would benefit greatly from such treatment. Fortunately some countries including the UK and the USA at least have adopted an approach of granting approvals on the basis of fairly broad classes of foods, and clearances are already in place for some of the most important candidate items. Elsewhere, Brazil (*22*) has taken the lead and has granted a blanket clearance for irradiation of all foods, even before the adoption of the most recent Codex standard. Hopefully other countries will soon follow this lead. For the present, existing regulations have allowed the expansion initiative that is so much in evidence in the USA, but the growth in usage would be expedited with a greater range of clearances. There is also a pressing need to broaden the range of clearances for the wide variety of packaging materials commonly used with food. An especially critical approval, expected sometime within 2003, is that for ready-to-eat (RTE) products. Beyond that, much work remains to be done to expand the existing clearances and especially to effect international harmonization of national regulations. In this regard, the agreements under the World Trade Organization (WTO) dealing with technical barriers to trade, and relating specifically to Sanitary and Phytosanitary (SPS) measures may provide a mechanism for hastening that harmonization (*23*).

A second major challenge concerns provision of adequate processing capacity to meet food industry needs. In these early days of commercialization, irradiation plants suitable for processing food are relatively few and far between, making it difficult for most manufacturers to get their products treated. Thus new plant capacity needs to be built in locations that can serve the requirements of the food manufacturers in a cost effective and convenient manner. Such new capacity will likely be provided through a number of different business arrangements, including dedicated in-plant systems, independent contract service facilities operating in a fee-for-service mode, systems installed at node points of the distribution system (such as cold storage warehouses and distribution centers), and undoubtedly many more. For the irradiation hardware, which is the heart of any irradiation facility, each specific site will select whichever technology option (x-ray, electron beam, gamma) best meets its particular processing needs. As the food irradiation industry expands, the need to lower costs, enhance effectiveness, increase reliability, and improve ease of operation will drive incremental design changes toward ever better systems.

Another challenge is that of labeling. Existing labeling requirements are generally acknowledged as being a deterrent to many processors who would otherwise use the process. In the USA the labeling issue is currently under review (*24*). Hopefully new regulations will allow use of wording that provides the information needed by consumers to make an informed choice, but which is not perceived as a warning sign.

There are marketing challenges. The good news is that there are many exciting successes, with excellent sales even at a significant price premium. However, experience to date has also shown clearly that some approaches work better than others. The greatest successes have been experienced by retailers who have approached the introduction of an irradiated product as they would other new products. This entails using a well thought out approach to ensure that knowledge of the advantages and benefits differentiating this product from others is effectively conveyed to their customers, and that adequate advertizing and other promotional support is provided. Undoubtedly the lessons learned in regard to what works and what doesn't will be used to guide future marketing initiatives.

A somewhat related challenge is that deriving from the activities of the activist groups that oppose this technology. These have been less and less effective as more and more retailers and processors join the ranks of those committed to offering their customers the choice of irradiated products. And most consumers by and large have chosen to ignore the rhetorical railings of the anti's, as evidenced by a demonstrated willingness to buy the products. Although future skirmishes with this constituency seem certain, it seems highly unlikely that the growing momentum in food irradiation will be deflected in any significant way by the activities of these self-appointed defenders of the public interest.

There are technical challenges also. Although food irradiation has been researched for decades, many technical challenges still remain, primarily on the food science side of the process. To be successful, irradiation of food must satisfy two objectives simultaneously, both of which are non-negotiable. First, it must effect the desired benefit, which usually means the killing of microbial pathogens or some unwanted pest. Second, product quality must not be compromised. Attainment of the required kill of microbe or pest is generally fairly easy to accomplish, since radiation inactivation of microorganisms has been extensively studied for decades, is relatively unaffected by differences in host product, and the effect of different processing conditions on radiation lethality to microorganisms is quite well understood. In general, killing a particular microorganism by irradiation in one food is similar to killing it in another food (*25, 26*), even though there may be some quantitative differences in killing kinetics. On the other hand, radiation effects on sensory quality are product specific in a qualitative sense and can be very strongly influenced by irradiation conditions (*27, 28*). Some products are easy to irradiate with excellent results, while others are more of a challenge. Thus, to ensure the necessary simultaneous attainment of both objectives, each product to be irradiated requires a validated treatment protocol, wherein the process variables influencing the irradiation response are carefully manipulated to ensure a successful outcome. Development of treatment protocols is an empirical process and requires the involvement of food scientists who are experts in the development of new products. Each product requires its own validated protocol, although many protocols may be very similar, if not identical. As irradiation becomes more widely accepted and used there will be a growing expenditure of effort in development of new protocols, as more companies work to adapt the process to their products. In that sense this will be no different than the continuing development of new cookbooks with new recipes for cooking food, even though food has been thermally processed since antiquity.

The challenges listed above are those that are more or less unique to the food irradiation industry in its present nascent state. Of course there are also those 'ordinary' challenges which confront any new business enterprise and which are an integral part of the business world. This includes access to capital, assembly of the teams of people with the necessary skills, market research, location and construction of plants, business arrangements, growth of the business, return on investment, and the like. In the coming years, as real world experience accumulates, dealing with these business questions in the food irradiation context will become easier. In the long run, the discipline of the market place will ensure that the needs of the industry will be met in the most effective manner. Eventually food irradiation will lose its status as a 'unique' type of business and become just another industrial activity. That will be the real indication of true success having been reached.

Summary and Conclusions

When all is said and done, the ultimate success of any new technology is determined by whether or not its adoption serves some useful purpose, providing a real benefit to society. In this regard it would appear that food irradiation is extraordinarily well positioned for success. It's main drivers, related to enhancement of both food safety and of trade in agricultural products, derive directly from basic human needs, and are inextricably intertwined in the workings of our food system. Modern, urban civilization is totally dependent on an advanced food system that is superbly efficient. Such a food system enables the specialization within society that impacts on every aspect of human activity and ultimately underlies all progress.' Unfortunately, this food system of ours has demonstrated an inherent vulnerability to the spread of various pathogens or pests that can adversely affect both consumers and ecosystems. An added twist is that the positive and negative features of the system derive from the very same attributes, to wit: mass production, central processing and widespread distribution. To counteract that vulnerability requires some means of differentially reducing the unwanted negative aspects without destroying the desired positive ones. Irradiation can do this, and thereby contributes directly to the enhancement of human health and welfare. The irreversibility of the structural changes associated with the urbanization of society ensures a permanence of the demand for the benefits of irradiation. While challenges exist in all the areas outlined above, the outline of a road around each of those challenges is already clear. The magnitude of the need and the accompanying opportunity is truly enormous, suggesting the potential of a continued expansion for several decades at least.

Closing Thoughts

Given the conditions and trends as described above, it is perhaps not too unreasonable to predict that within the next decade or so irradiation will finally take its rightful place, along with pasteurization, immunization and chlorination, as the "fourth pillar of public health" (*29*). There could be no finer tribute to those many many workers who labored through the decades developing the scientific base for this technology than for irradiation to finally attain a status where it is genuinely contributing on a daily basis to the health and welfare of ordinary people.

> *"The great French Marshall Lyautey once asked his gardener to plant a tree. The gardener objected that the tree was slow growing and would not reach maturity for 100 years. The Marshall replied, " In that case, there is no time to lose; plant it this afternoon. "*
>
> John F. Kennedy

It's been almost exactly 100 years now since the first moves towards food irradiation were suggested by early visionaries; perhaps that seedling is finally reaching maturity.

Acknowledgements

Thanks to Paisan Loaharanu and Jiri Kotler for their helpful comments.

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