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# IRRADIATION OF FOODS AND ITS EFFECT ON LIPIDS

Gerhard Maerker

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## 1. INTRODUCTION

Two patents on the use of radiant energy to preserve foods by the elimination of spoilage organisms were issued as early as 1905, only nine years after the discovery of radioactivity by Henri Becquerel [1]. However, intensive studies of the irradiation of foods were not initiated until the early 1950s, when radiation sources became more widely available. Pioneering food irradiation research programs, such as the one undertaken under the auspices of the U.S. Atomic Energy Commission in 1950, as well as President Eisenhower's Atoms for Peace program a few years later, were motivated in part by a desire to find new uses for radioactive by-products of the nuclear power initiative [1].

Since then, ionizing radiation has been proposed for (1) insect disinfestation of grain; dried spices, vegetables, or fruits; and fresh fruits; (2) inhibition of sprouting in tubers and bulbs; (3) alteration of post-harvest ripening and senescence of fruits; (4) inactivation of protozoa or helminths in meats and fish; (5) elimination of spoilage microorganisms from fresh fruits and vegetables; (6) pasteurization or sterilization of dried spices and vegetables; (7) extension of shelf-life of meats, poultry, fish, or shellfish; (8) elimination of bacterial pathogens from meats, poultry, fish, or shellfish; and (9) sterilization of foods and feeds [2].

Ionizing radiation has a long history of successful commercial use in the sterilization of medical supplies and in chemical applications. Its use in food processing also made notable progress

during the second half of this century, so that by the end of 1993, 37 countries had approved more than 40 foods and food groups for irradiation [3]. Nevertheless, the process of food irradiation and its products have become controversial in some countries. On the one hand, benefits such as the destruction of food-borne pathogens, and thereby the reduction in illnesses resulting from the ingestion of food, are well recognized. On the other hand, there is the expressed fear that the process might induce consumers to eat food that should be rejected, or that the process might make food radioactive or, at the least, that irradiation might generate in food some unknown factor that is harmful to health [4].

Because of consumer concerns, some governments have been reluctant to approve certain food irradiation processes until it has become possible to develop analytical methods capable of demonstrating reliably, after the fact, whether or not food has been irradiated (see below). In this connection it is interesting to note that a worldwide community of scientists has searched irradiated foods for more than 40 years to detect products that are uniquely generated by the irradiation process. In a few isolated cases, some progress toward this goal has been made, but as yet there is no internationally accepted method that can be used with assurance in field laboratories and that can be applied to a wide variety of foods. The relative futility of the search for unique radiolysis products is testimony to the triviality of chemical changes produced in foods by ionizing radiation, given the current sophistication and power of the analytical tools and methods available to the modern scientist. This is not to say that chemical changes do not occur. They do, but they occur in unirradiated and irradiated foods alike, although perhaps by different mechanisms, at different rates, to different degrees, or resulting in different product ratios.

Extensive literature exists describing studies that demonstrate the wholesomeness and safety of irradiated foods [2, 4, 5]. Reported investigations include, among others, nutritional, genetic toxicological, teratogenic, and extended multigenerational feeding studies on several species of animals. No adverse effects due to irradiated foods were discovered.

It is also important to note that the process of irradiation, as practiced, is incapable of imparting radioactivity to foods, and that irradiated foods are not radioactive. Food cannot become radioactive from exposure to gamma rays from cobalt 60 or cesium 137, from X-rays of 5 MeV or lower energy, or from accelerated electrons with energy levels of 10 MeV or less [6].

If irradiation is to be used more widely as a food-processing procedure, it is clear that it will have to compete in the marketplace with other food-processing methods, both technically and economically. Therefore, for this technology to be appropriate, it must control target spoilage and pathogenic organisms, and it must not adversely affect the wholesomeness of the food product. Any effects on the nutritional value and the organoleptic properties of the treated food must be within acceptable limits. The marketplace will define what sensory changes are acceptable, but nutritional changes must be defined by chemical and biological analyses of the treated product, because it will be consumed.

## 2. BASIC PRINCIPLES

The types of ionizing radiation most frequently used in food processing are  $\gamma$ -radiation and electron beams. To be suitable for this purpose, the energy of the radiation must be sufficient to exceed the ionization potential of target atoms or molecules, but insufficient to interact with the nuclei of such targets.

Electron beams are generated by electron accelerators. Linear electron accelerators are used to impart to electrons the necessary energy to enable them to penetrate the medium (food) in a coherent beam. These machines have the advantage that they can be generated at the desired energy level, and that they can be shut off when not in use [7]. Their disadvantage in food processing is their shallow depth of penetration in comparison with  $\gamma$ -radiation. Ramler [8] has discussed linear accelerators that can be used for industrial purposes.

$\gamma$ -Rays are electromagnetic waves emitted by unstable isotopes during radioactive decay.  ${}_{55}\text{Cs}^{137}$  and  ${}_{27}\text{Co}^{60}$  are the most commonly used and the most readily available among the radioactive

sources for food irradiation. Both are produced in nuclear reactors. Cesium 137 is a product of the fission process, has a half-life of about 30 years, emits  $\gamma$ -radiation with 0.66 MeV energy, and decays to  $_{56}\text{Ba}^{137}$ . Cobalt 60 is not a fission product but is produced in nuclear reactors by the absorption of a thermal neutron by  $_{27}\text{Co}^{59}$  [9]. Cobalt 60 has a half-life of 5.27 years and emits  $\gamma$ -radiation of 1.17 MeV and 1.33 MeV energy. It also emits an electron with a maximum energy of 0.31 MeV and an average energy of 0.094 MeV. Cobalt 60 decays to  $_{28}\text{Ni}^{60}$  [9]. The electron volt (eV) is still the commonly used energy unit for radiation, where  $1 \text{ eV} = 1.602 \times 10^{-19} \text{ J}$ .

The quantity of radioactivity is measured in terms of curies (Ci), where  $1 \text{ Ci} = 3.70 \times 10^{10}$  disintegrations per second, or in Becquerels (Bq), which is the amount of radioactivity resulting from one nuclear transformation per second. For the purpose of food processing, however, the more relevant quantity is the amount of energy absorbed: the dose. The generally accepted unit of dose was formerly the rad (radiation absorbed dose), where  $1 \text{ rad} = 10^{-5} \text{ J}$ . The rad has fallen into disuse and has been supplanted by the Gray (Gy), where 1 Gy is the unit of absorbed radiation equal to 1 J of energy absorbed by 1 kg of material. It follows that  $1 \text{ Gy} = 100 \text{ rad}$  and  $1 \text{ kGy} = 10^3 \text{ Gy}$ .

The process of ionization by radiation is seen as a series of discrete energy transfer steps called the Compton effect. The ionization potential of most atoms is in the range of about 4 to about 20 eV [10], depending on the specific atom, but the energy of the incident radiation, photons or electrons, is far in excess of that required to produce ionization or electronic excitation. The encounter of the incident photon with an atom of the target medium causes the formation of an ion pair with the ejection of a valence electron from the target atom. The incident photon transfers some of its energy to the ejected electron. The net result of this collision is that the photon, now a secondary photon with diminished energy and a new directional pathway, continues until it encounters further atoms, while the ejected electron transfers some of its energy to another atom, where it ejects a valence electron that continues on. Thus through a series of many discrete energy transfers, the energy of the incident

photon is imparted upon the target medium along a series of spurs or tracks. Some of the energy transfers result in the formation of electronically excited atoms or molecules, rather than in ionizations. The process of energy transfer along spurs continues until the rays or their products are no longer able to transfer energy.

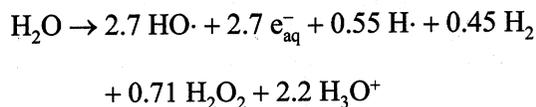
The electronically excited or ionized atoms or molecules are chemically unstable species and must undergo changes to gain stability. The primary effect of radiation on atomic or molecular species is called the *direct effect* [10] and includes subsequent changes that the species undergoes to gain stability. In many cases, however, the primary species produced interacts with a chemical species previously unaffected by the radiation. This is called the *indirect effect*. The latter depends to some extent on the ability of the primary species to move about, so that it can collide with the secondary target molecule. Hence, viscosity of the medium is important, as is the concentration of the secondary target.

Water is a major component of many foods, ~85% in fruits and vegetables, ~70–75% in meat and poultry, and ~75–85% in seafood [7]. In the irradiation of these foods, water absorbs a large part of the radiation energy, and an understanding of the principles of the radiolysis of water contributes significantly to an appreciation of the radiation chemistry of these foods.

It is generally agreed [7, 10, 11] that the radiolysis of water results in the generation of six principal products: H·, HO·,  $e_{aq}^-$ , H<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, and H<sub>3</sub>O<sup>+</sup>. According to Gauduel [12], the timing of events following the absorption of radiation by water has been measured by femtosecond techniques and ranges from the 10<sup>-16</sup> s required for the formation of excited or ionized water to the 10<sup>-7</sup> s needed for the formation of molecular products in the spur and diffusion out of the spur. Both the hydrated electron ( $e_{aq}^-$ ) and the hydroxyl radical (HO·) are highly reactive species whose half-lives are <10<sup>-9</sup> s [13]. They react with various chemical constituents at diffusion-controlled rates.

The quantities of radiolytically produced species are generally expressed in terms of *G*-values, which are the number of transformed molecules per 100 eV of absorbed radiation. *G*-values

are usually less than 10 [7]. For the radiolysis of water, the  $G$ -values of the principal products are generally accepted to be [7]



but slightly different values have been reported by von Sonntag [14] and by Simic [15]. The difficulties encountered in attempts to determine the primary yields of  $\text{H}\cdot$ ,  $\text{HO}\cdot$ , and  $e_{\text{aq}}^-$  have been described by Jonah [16]. The latter also comments that there is a lack of evidence for the existence of the excited state of water in radiolysis.

The hydroxyl radical is a strong oxidizing agent that oxidizes metals and adds to carbon-carbon double bonds [17]. The rate constant for the abstraction of hydrogen atoms from aliphatic carbon compounds by  $\text{HO}\cdot$  has been determined [18]. The hydrated electron and the hydrogen atom are both strong reducing agents, their  $E^\circ$  being  $-2.8$  V and  $-2.0$  V, respectively [15].

In the irradiation of dilute ( $<0.1$  M) aqueous solutions most of the radiation energy is absorbed by water. The water radiolysis products then may undergo secondary reactions with the solute molecules, i.e., indirect reactions predominate [10]. At higher concentrations ( $>1$  M) direct reactions become important. As the number of solutes increases, each has a diminishing opportunity to encounter a primary water radiolysis product, so that the number of secondary radiolysis products becomes larger, but the concentration of each becomes smaller. Urbain [10] has pointed out that this is exactly the situation that exists in foods in which water is a major component. The very large number of food components can be expected to yield very many radiolytic products, each at vanishingly low concentrations. Undoubtedly this situation is modified by the presence in some of the food components of molecular architecture that may be more susceptible to attack by radiation or primary radiolysis products than others. Furthermore, some polar components of foods may be in closer contact with the aqueous domains of the food than are some nonpolar components.

In addition, the temperature at which the food is irradiated plays an important role with foods of high water content. When the water is in the liquid state, the primary radiolysis products are mobile and have an opportunity to interact with other food components. Freezing the food limits mobility and thereby limits indirect action.

The dose rate, i.e., the rate at which radiation is absorbed by the target medium, can be varied in some situations. Sometimes it can influence the relative amounts of radiolysis products generated [10]. At high dose rates the concentration of primary free radical products may increase to the extent that recombination or radical-radical termination reactions become important at the expense of indirect products.

In the absence of water or other diluents, the direct effect is the predominant result of irradiation. In this situation, the primary radiolysis products gain stability by an intramolecular mechanism leading to stable products. For lipids, for example, it has been demonstrated [7] that the energy of ionizing radiation far exceeds the bond dissociation energy of any of the bonds of a lipid molecule. It will be shown later that the irradiation of lipids does indeed result in the formation of fragments. Nawar [19] suggested that the primary products of the irradiation of lipids are molecular ions and electronically excited molecules, which then undergo cleavage in secondary, stabilizing reactions.

Urbain [10] considered in detail the possibilities of generating induced radioactivity in foods. He concluded that even at sterilization doses the amount of induced radioactivity produced in meat is not more than  $10^{-7}$  of that present in unirradiated meat. He calculated that the induced radioactivity constitutes about  $10^{-6}$  Bq, or one disintegration per week.

### **3. MODEL SYSTEMS**

#### **3.1 Fatty Acids and Neutral Lipids**

One of the principal purposes of radiation processing of foods is to effect a decrease in the food's population of microbes, parasites, or insects. Neat fats and oils, however, are rarely con-

taminated with living populations of such organisms [10], and other beneficial effects of radiation processing of fats and oils have not been described. Hence, in commercial practice the exposure of lipids to ionizing radiation usually occurs when such lipids are a part of a more complex food product to be processed. Lipids occur in foods in different forms. They may be present as separate domains, as, for instance, in adipose tissue, or they may appear as structural components in proximity to an aqueous phase, as, for example, in cell membranes. Furthermore, they may exist in combination with other classes of food components, as in glycolipids or in lipoproteins. In all of these instances, the degree and manner in which lipid structures are affected may depend on the radiosensitivity of nearby nonlipid molecules.

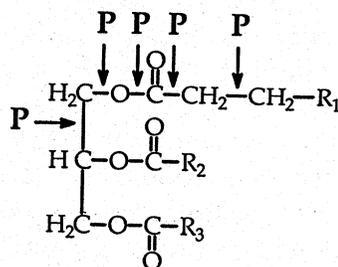
The chemical effect that ionizing radiation has on lipid components of food may be influenced by the microenvironment in which these components dwell. For example, if the lipid components are in close contact with an aqueous phase, much of the radiation energy may be absorbed by the water to form primary water radiolysis products, which then interact with the lipid via the indirect effect. It will be pointed out later that other parameters influence the products formed by the interaction of ionizing radiation with food lipids. Such factors are the dose, dose rate, temperature, the physical state of the lipids, and the presence of oxygen.

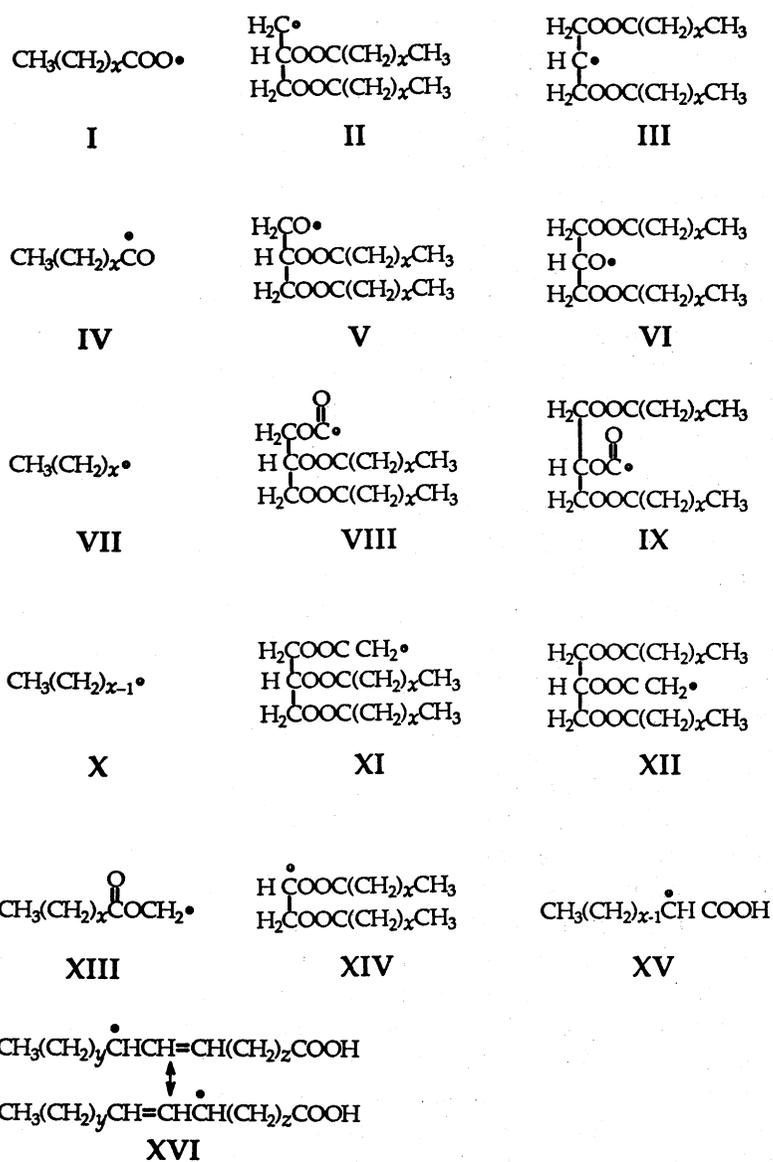
Because of the extremely complex and variable nature of food systems, they represent inordinately difficult substrates for research. As mentioned earlier, the chemical energy represented by the incident radiation is distributed among many of the numerous chemical entities present, affecting each to a minute degree, and giving rise to a very large number of radiolytic products, each in vanishingly low amounts. For this reason, early researchers studied the effect of ionizing radiation on lipids in model systems. The substrates in these experiments usually were highly purified fatty acids or triacylglycerols. The knowledge gained from the study of these model systems was then applied to the radiolysis of food lipids.

Results of studies of the radiolysis of fatty acid and neutral lipid model systems have been presented in a number of excellent reviews [7, 10, 19–23] and will be summarized here only to present an overview. Additional studies will be described in somewhat greater detail to bring the reader up to date.

Extensive investigations have been carried out on the irradiation of synthetic triacylglycerols in which all three fatty acid residues are the same. In particular, tributyrin, tricaproin, and tripalmitin were exposed to doses of  $\gamma$ -radiation up to 500 kGy and higher, at several temperatures and in the presence or absence of air. Numerous radiolytic products were identified and were shown to be the result of the cleavage of one or more bonds of the parent molecule. Nawar [19] classified these products into three groups. Primary radiolytic products are defined as those formed by the scission of one bond of the triacylglycerol, followed by abstraction or loss of one hydrogen atom. Recombination products are those formed by the combination of free radicals formed initially. Secondary products are those believed to be the result of more than one bond cleavage in the same parent molecule or of the decomposition of primary products. The probability of such secondary events is rather low, and such products are expected to be formed, and indeed were found to occur, in relatively low concentrations.

Quantitative analysis of triacylglycerol radiolysis products demonstrated that bond cleavage in the molecule showed a non-statistical distribution. Bonds in the vicinity of the carbonyl





*Scheme 1.*

groups cleaved preferentially, and bonds on both sides of the acyl oxygen were most frequently affected [19]. However, bond cleavage elsewhere in the molecule also occurs.

Principal bond cleavage positions (P) are indicated in Structure 1 above. Radicals that are believed to be formed as a result of bond scission were listed by Nawar [19] as shown in Scheme 1.

Among the principal primary radiolysis products of saturated triacylglycerols are a free fatty acid and an aldehyde that have the same number of carbon atoms as the parent acid. These are believed to be the result of hydrogen abstraction by radicals I and IV, respectively. Other prominent primary radiolysis products are the  $n - 1$  alkanes and alkenes, derived from radical VII; propanediol diesters, formed from II and III; and 2-alkylcyclobutanol, hypothesized to result from the cyclization of a radical cation generated by the interaction of  $\gamma$ -rays with a carbonyl oxygen [19].

Recombination products include hydrocarbons with carbon numbers exceeding those of the alkyl chain of the parent fatty acid. These are believed to be formed from the combination of radical VII with itself or with shorter chain alkyl radicals. Other examples of recombination products are ketones, such as may be formed by the termination reaction of two type IV radicals or the combination of radicals IV and VII. Esters arise from the reaction of radicals I and VII or X and XIII. Many other radical recombination products have been discovered among the radiolysis products of saturated triacylglycerols.

Much of the knowledge of the effect of ionizing radiation on saturated triacylglycerols has been gained from a systematic investigation of the radiolysis of synthetic tricaproin irradiated to 60 kGy [24–27]. In these studies 28 recombination products were identified. The mechanism of formation of many of the radiolysis products was supported and confirmed by an examination of the radiolysis of tributyrin labeled with deuterium in the glycerol backbone [28]. Additional radiolysis products, including butanetriol triesters, erythritol tetraesters, and polyglycol polyesters, were identified after tributyrin was irradiated to 500 kGy under vacuum at room temperature [29]. Mass spec-

trometry provided evidence that tributyrin and tripalmitin irradiated to 250 kGy under vacuum at 25°C formed yet further products, such as triacylglycerol adducts including  $\alpha$ -branched alkyl-substituted compounds [30]. Electron spin resonance (ESR) spectroscopy gave evidence for the transient formation of several of the radicals formed initially during the irradiation of lipids [31].

Free fatty acids form essentially the same radiolysis products as the corresponding triacylglycerols [10]. However, there are quantitative differences in the relative amounts of products formed. For example, palmitic acid gives rise to about 20 times the amount of  $n - 1$  alkane produced from tripalmitin [32]. Wu and Howton [33] irradiated crystalline stearic acid under nitrogen to the extremely high doses of 1 and 4.5 MGy and found the usual radiolysis products, but also unexpectedly high amounts of tetratriacontane ( $C_{34}$ ), diheptadecyl ketone, and an  $\alpha,\alpha$ -dimer of stearic acid. They attributed the relative ease of formation of these dimeric products to heptadecyl radicals formed by double decarboxylation of the hydrogen-bonded stearic acid "dimer" existing in the crystalline acid.

In unsaturated fatty acids, the double bond represents an additional site for interaction with radiant energy. The radiolysis of oleic acid, triolein, and alkyl oleates has been reported, but studies of trilinolein and of pure triacylglycerols with higher degrees of unsaturation have not been described. In a comparison of the radiolytic compounds from palmitic and oleic acids and from tripalmitin and triolein [34], the saturated substrates gave larger amounts of the most abundant primary and recombination products; but additional products involving the double-bond site were not reported. Irradiation of ethyl palmitate, ethyl  $\alpha$ - $d_2$ -palmitate, and ethyl oleate revealed that in the saturated compounds adduct formation occurred mainly in the  $\alpha$ -position, and ethyl oleate yielded monounsaturated and diunsaturated dimers of both the vinylic and allylic types [35, 36]. Irradiation of mono-, di-, and tripalmitoylglycerol [37] yielded products predicted by the proposed mechanism [19].

It is generally agreed that irradiation in the presence of oxygen, or followed by storage in air, accelerates the oxidation of

lipids. This is as expected, since ionizing radiation can be viewed as the initiator of classical free radical autoxidation [38]. Furthermore, the hydroperoxides formed by the interaction of oxygen with the primary radicals formed in radiolysis are themselves subject to homolytic cleavage by radiation [39], giving rise to two new radicals. Although the acceleration of lipid oxidation by irradiation in the presence of oxygen is well established, it is less clear to what extent this acceleration takes place. Moreover, the effects of dose and dose rate in this process have not been well defined.

As food processing techniques, irradiation and thermal treatment share the common purpose of shelf-life extension by decreasing the population of microorganisms. A comparison of the lipid products from the two processes is therefore particularly relevant and has been made [40]. With regard to model compounds, a detailed comparison was made of products from heating tricaproin at 270°C for 15 h under vacuum and from irradiating the same compound to 60 kGy, also under vacuum. The product compositions from the two processes were qualitatively almost identical (no 2-alkylcyclobutanone was formed by heating), but there were differences in the relative amounts formed.

### **3.2 Polar Lipids and Synthetic Membranes**

The ability of polar lipids, such as phospholipids, to form closed synthetic vesicles has been recognized for some time [41, 42]. Such vesicles, generally known as liposomes, have been used extensively as models for biological membranes and especially as models for the study of lipid peroxidation in such membranes [43, 44]. Liposomes can be prepared as unilamellar or as multilamellar vesicles, and their sizes and properties can vary widely, depending on their composition and the technique used for their preparation [45–50].

Biological membranes, as well as their synthetic models, are pictured to contain a continuous lipid bilayer made up of phospholipid molecules. The latter are oriented in such a fashion that the phosphorus-containing headgroups face the outer and

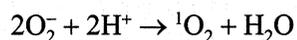
inner aqueous phases, and the fatty acid hydrocarbon chains form a hydrophobic domain within the membrane.

In view of the fact that phospholipid molecules form synthetic vesicles spontaneously (they also form micelles, to be discussed later), it is not surprising that the bulk of the research on the effect of ionizing radiation on phospholipids has been carried out on liposomes. It is unexpected, however, that the research on such model systems has revealed very little information on the effect of the radiation on the polar headgroups. The latter, containing nitrogen and phosphorus, are electron rich and are in intimate contact with water, and hence its radiolysis products [19, 21].

Phospholipids in biological membranes often contain unsaturated and polyunsaturated fatty acids. In several of the studies on the effect of radiation on synthetic membranes, the model systems were constructed from phospholipids extracted from egg yolk lecithin or soybean lecithin, both of which contain substantial amounts of polyunsaturated fatty acids. The strategy pursued was to measure the radiation effect by the free radical-induced chemical changes in the fatty acid hydrocarbon chains.

Radiation-induced lipid peroxidation and changes in membrane permeability were measured in liposomes generated from soybean lecithin that contained 55% linoleic acid [51]. Lipid peroxidation was measured by the determination of malondialdehyde (MDA) by a modification of the thiobarbituric acid test [52], and the integrity of the membrane was determined by glucose efflux. Both MDA formation and glucose efflux varied linearly with dose between 20 and 100 Gy at dose rates of 0.05 and 2.1 Gy/min. Free radical scavengers inhibited both MDA formation and glucose efflux. The authors suggested that the OH radical is important in the modification of membrane permeability. The addition of cholesterol or cepharanthin, an alkaloid known to stabilize membranes, to liposomes prepared from the same soybean lecithin, decreased glucose efflux [53]. When unstabilized liposomes were irradiated to 2.5 kGy, conjugated diene, peroxide value, TBA value, and carbonyl value all increased linearly with dose [54].

Petkau and Chelack [55] irradiated liposomes with X-rays or with  $\gamma$ -radiation from Cs 137 and measured lipid peroxidation by an increase in conjugated diene absorption at 232 nm. Large increases in conjugated diene were observed when model membranes were irradiated. A large part of this increase was ascribed to superoxide anion,  $O_2^-$ , since bovine superoxide dismutase, when present in the medium, prevented 80% of that increase. Superoxide anion arises from the rapid reaction of the water radiolysis product  $e_{aq}^-$  with oxygen. The following reaction then ensues:



Singlet oxygen is known to react with linoleate about 1500 times faster than ground state triplet oxygen [38]. Singlet oxygen is well known to be produced in photosensitized lipid peroxidation in which light energy is transferred to ground-state (triplet) oxygen via a photosensitizer. Singlet oxygen adds directly to carbon-carbon double bonds by a concerted "ene" addition to produce hydroperoxides [56].

Petkau and Chelack [55] carried out some studies in which irradiation of liposomes was conducted under  $N_2O$ , which converts  $e_{aq}^-$  to  $\cdot OH$ , thus in effect doubling the OH radical concentration. The amount of conjugated diene formed under  $N_2O$  was much less than that formed under air and only slightly more than that formed under  $N_2$ . The authors concluded that  $\cdot OH$  was not significantly involved in fatty acid peroxidation.

An important observation was that diene conjugation increased with decreasing dose rate when membranes were irradiated to a constant dose [55]. This was explained on the basis that irradiation initiates autoxidation chain reactions, which proceeded further at lower dose rates in the longer time required to reach a given dose.

Other authors [57, 58] confirmed the inverse relationship of dose rate and liposome peroxidation. However, there is considerable disagreement on the identity of the water radiolysis product that is responsible for the initiation of the autoxidation process that damages model membranes. O'Connell and Garner

[58] irradiated small unilamellar vesicles, measured lipid peroxidation by the formation of malondialdehyde, and found that superoxide dismutase inhibited the oxidation process only slightly. On the other hand, they presented strong evidence in support of their hypothesis that  $\cdot\text{OH}$  is responsible for initiating radiation damage in liposomes. Other research [59–62] substantiates that viewpoint.

A number of compounds protect liposomes from oxidation, at least in part. Among these are  $\alpha$ -tocopherol, reduced glutathione, and cysteamine [57], as well as mannitol and sodium benzoate [58]. The chloride anion of sodium chloride as well as 5-nitro-2-furaldehyde have also been found to protect liposomes against radiation damage [64]. Fatty acids in solution are protected against diene conjugation by the free radical scavengers ethanol and sodium formate [59].

Although lipid peroxidation in liposomes has been most frequently assessed by measurement of diene conjugation and production of malondialdehyde, other methods have been reported recently. Sprinz and co-workers [63] used nuclear magnetic resonance (NMR) spectroscopy to measure the penetration of  $\text{Eu}^{3+}$  into irradiated liposomes as well as to detect damage to lipid molecules in those vesicles. Laser Raman spectroscopy has been utilized to study the effect of ionizing radiation on thermal transitions in liposomes composed of dipalmitoylphosphatidylcholine and polyunsaturated fatty acids [64].

Fatty acids and other bipolar molecules form aggregates when their concentration in aqueous solution exceeds a characteristic limit. The aggregates are called *micelles* and the characteristic limit is called the *critical micelle concentration* (CMC). Structurally, micelles are different from liposomes, in that they do not contain a distinct inner aqueous phase. The surface of micelles contains the charged headgroups, whereas the interior of these aggregates consists essentially of a hydrophobic phase formed by the hydrocarbon tails of the fatty acid molecules [65].

Yau and Mencl [66] studied the peroxidation of fatty acids induced and promoted in micelles by ionizing radiation. They measured diene conjugation and the production of TBA-active

materials and observed the same inverse relationship between dose rate and peroxidation at constant dose observed by others in liposomes (see above). Furthermore, several investigators found that the peroxidative effect induced in polyunsaturated fatty acids increases drastically above the CMC [59, 60, 67]. This has been interpreted to mean "that the propagation step (chain reaction) and not the initiation step determines the radiosensitivity of the different fatty acids in the single component micelles" [60].

Very little research has been performed to define the mode of interaction of ionizing radiation with individual phospholipids of defined molecular structure. This is probably due, in part, to the fact that highly purified compounds of this type have become commercially available only in recent years, albeit at considerable cost.

An early source [68] reported that lecithin of an unspecified origin or purity, or a related hydrogenated lecithin, interacted with ionizing radiation to yield a free fatty acid, a lyso compound, and choline phosphate. The source of this information is unclear, since no experimental details or literature sources were given. Irradiation of dipalmitoylphosphatidylethanolamine [21, 40, 69] to 500 kGy resulted in the formation of the volatile hydrocarbons, aldehydes, ketones, and esters expected from the nonpolar acylglycerol portion of the molecule. However, the amounts of products resulting from radiolysis near the carbonyl group were sharply reduced. The authors pointed to the presence of electron-rich functional groups in the polar headgroup of the phospholipid as a possible cause for the diminished amounts of radiolytic products stemming from the nonpolar portion of the molecule. Indeed, they were successful in the thin-layer chromatographic (TLC) separation of such compounds as lysophosphatidylethanolamine and phosphorylethanolamine. These results are confirmed by some electron spin resonance (ESR) studies [31] in which a radical of phosphorylethanolamine was detected.

A series of individual phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, and phosphatidylglycerols containing either saturated or unsaturated fatty acid chains was

irradiated to about 10 kGy in aqueous suspension [70, 71]. High-performance liquid chromatography (HPLC) was used to isolate various products such as dipalmitoylphosphatidic acid and lysophosphatidylcholines. The structures of these compounds were confirmed by mass spectrometry.

### 3.3 Sterols

Cholesterol and the phytosterols are usually grouped with the lipids. They are extracted from tissue with the lipids and are isolated together with other unsaponifiable compounds. They contain at least one double bond and hence are subject to chemical or enzymatic oxidation. Cholesterol and the principal plant sterols have identical chemical structures with regard to their polycyclic nucleus of four fused rings and differ principally in the structures of their aliphatic side chains.

Cholesterol is a component of the cell membrane of animal tissues. Cholesterol autoxidation has been recognized since the beginning of the twentieth century, but its study intensified and became systematized in the 1960s [72]. Prevailing knowledge of cholesterol autoxidation was summarized in 1987 [73, 74].

More than 70 cholesterol oxidation products, or "cholesterol oxides," are known, and a few of these have been reported to have adverse effects on human health. Some of these effects are cytotoxicity [75–78], angiotoxicity [79], mutagenicity [80–84], carcinogenicity [85–87], and others [88–93]. Several of these biological activities have led researchers to speculate that a link may exist between ingested cholesterol oxidation products and coronary heart disease [94].

The principal product of the autoxidation of cholesterol in dispersions, in solutions, or in liposomes is 7-ketocholesterol (3 $\beta$ -hydroxycholest-5-ene-7-one). Other prominent autoxidation products include 7 $\alpha$ - and 7 $\beta$ -hydroxycholesterol (cholest-5-ene-3 $\beta$ ,7 $\alpha$ -diol and cholest-5-ene-3 $\beta$ ,7 $\beta$ -diol, respectively) and  $\alpha$ - and  $\beta$ -epoxide (cholesterol 5 $\alpha$ ,6 $\alpha$ -epoxide and cholesterol 5 $\beta$ ,6 $\beta$ -epoxide, respectively). Typically, the relative amounts of 7-ketone:7-hydroxy:5,6-epoxide formed in cholesterol autoxidation in aqueous media have been about 10:5:1 [95–97].

$\gamma$ -Irradiation of cholesterol in aqueous media also generates 7-ketocholesterol, the anchimeric 7-hydroxycholesterols, and the 5,6-epoxides as the principal products. However, the ratios of the products are considerably different from what they are in autoxidation [98, 99]. For example, whereas the ratio of 7-ketone to epoxides is about 10 in autoxidation, it is less than 1 after cholesterol has been treated with ionizing radiation. Furthermore, some of the cholesterol oxides formed by irradiation initially appear to exhibit some instability to further irradiation [100, 101]. This instability causes the generation of two compounds not normally observed among the autoxidation products: 6-ketocholestanol and 7-ketocholestanol. These are cholesterol derivatives that lack the double bond of the parent compound [101]. In addition,  $\gamma$ -irradiation causes the formation of significant amounts of A-ring oxidation products [102]. Some of these cholesterol oxides have been reported to be products of the enzymatic oxidation of cholesterol, but they are not usually found among the compounds formed by autoxidation.

## **4. FOOD LIPIDS**

### **4.1 General Considerations**

Intensive research into the potential of ionizing radiation as a means of food processing began in the 1950s, when sources for such radiation became more generally available. In the 1960s and the 1970s about 20 countries completed the necessary investigations and the legal actions to clear irradiated foods for human consumption [103]. Most of these clearances were for sprout inhibition and for control of insect infestation, but a few countries, among them Canada, South Africa, The Netherlands, and the Soviet Union, also cleared some poultry and some seafoods, at least provisionally. Meanwhile, the scientific literature gave evidence of increased research activity delving into the effects of ionizing radiation on a variety of foods.

The state of the knowledge of the radiation processing of foods in early 1981 has been capably and thoroughly summarized by Diehl [104]. From this report it is clear that there is a substantial

difference between the radiation chemistry of pure substances and that of the same substances when they are components of complex food systems. The differences, however, are mostly quantitative, rather than qualitative.

It has already been pointed out that the multiple components of complex food systems may differ in their affinity for interaction with ionizing radiation or its primary products. As a result, some food components act as radiation protective substances. Compounds, such as cystine, cysteine, ascorbic acid, and others, are free radical scavengers, so that radiation-induced changes in a foodstuff may not be evenly distributed. Some investigators have attempted to take advantage of this situation by the use of additives to protect food from chemical changes caused by radiation, but this approach has not been altogether successful.

The review by Diehl [104] adequately describes the effect of processing parameters on the results obtained, and some of the points are worth repeating here to provide background information. Irradiation of meat to 60 kGy produces a very large number of volatiles in low concentration. The straight-chain hydrocarbons have been demonstrated to result from the radiolysis of meat lipids, whereas sulfur compounds and alkylbenzenes stem from the irradiation of meat proteins. Organoleptic properties deteriorate with increasing dose. Irradiation of meat at cryogenic temperatures improves the flavor profile but may require higher doses to eliminate bacteria. Proteolytic enzymes of meat and poultry are fairly resistant to radiation damage and must be heat-inactivated before irradiation to preserve the texture of the processed meat on storage.

The question of whether irradiation under the exclusion of oxygen produces beneficial results had not been resolved by 1981. Much depends on the composition of the food, the temperature at which it is irradiated and stored, and other variables. Foods that have a high content of unsaturated and polyunsaturated lipids seem to suffer accelerated peroxidation when they are irradiated in the presence of air, as well as when they are stored in air subsequent to irradiation.

## 4.2 Meat and Poultry

An essential goal of the radiation treatment of meats and poultry is the achievement of an increased shelf life of the food by the reduction of the microbial population. The growth of microorganisms present in and on the surface of meats and poultry gives rise to effects, such as changes in flavor, odor, and appearance [105]. Furthermore, the presence of pathogens may present a serious health problem to the consumer.

Low-dose irradiation (<10 kGy) is sufficient to reduce the population of vegetative bacteria drastically [105, 106], but it is also enough to accelerate lipid oxidation during irradiation and subsequent storage and thus contribute to off flavors (see below). Thayer [107] has discussed the application of low doses of  $\gamma$ -radiation to eliminate or greatly reduce populations of microorganisms and significantly extend the shelf life of poultry pork and beef while preserving the nutritional and perceptible properties of the products.

Much of the research on the effect of ionizing radiation on meat and poultry in the 1980s and early 1990s was carried out on chicken. Katta *et al.* [108] treated broiler chickens in a commercial irradiation facility with up to 3.0 kGy doses of  $\gamma$ -radiation at 3.0°C and found that more than 99% of the microorganisms were destroyed at doses ranging from 1.5 to 2.0 kGy. They did not discover any changes in the fatty acid composition, except that palmitic acid decreased and oleic acid increased. Rady *et al.* [109] also found only minor changes in the fatty acid profiles after they irradiated chicken muscle to 1 to 10 kGy at a temperature of -20°C in either air or vacuum, although they did see slight decreases in the unsaturated fatty acids of polar lipids.

Other researchers took a different approach to the detection of the effect of radiation on food lipids, especially those of meat and poultry. Rather than trying to measure the relatively minor changes in fatty acid profiles caused by low-dose irradiation, i.e., small differences between large numbers, they attempted to find unique radiolysis products of lipids. Or they searched for and tried to measure radiation-caused increases of components normally present in trace amounts. Another ap-

proach was to apply very large doses of radiation to the food, so that changes in lipids or the appearance of their radiolysis products presented fewer analytical difficulties. Some combined both of these approaches.

Merritt *et al.* [110] made a quantitative comparison of major radiolysis products obtained by the irradiation of chicken, beef, pork, and ham to 30 to 90 kGy at  $-40^{\circ}\text{C}$ . Major products resulting from the radiolysis of meat lipids were separated into two groups: volatile compounds (hydrocarbons up to  $\text{C}_8$ ) and nonvolatile compounds (hydrocarbons up to  $\text{C}_{17}$ , hexadecanal and propanediol diesters). The yields of all of these most prominent products varied directly with dose and with the fat content of the food. Individual hydrocarbons varied linearly with the content of the proposed precursor fatty acids in the meats. For example, pentadecane varied with palmitic acid content, heptadecene with oleic acid content, and heptadecadiene with the linoleic acid content of the meat. This work and preceding reports [111–113] form the basis for a method for the detection of irradiated meat (see below).

A second group of workers [114] simplified the isolation procedure for the hydrocarbons somewhat but confirmed the results obtained by Merritt *et al.* [110], when they measured radiolytically generated hydrocarbons in several meat products after application of doses of up to 10.2 kGy. Earlier they had obtained similar results when they measured  $\text{C}_{15}$  and  $\text{C}_{17}$  hydrocarbons produced by the irradiation of frog legs and compared the results with those obtained by an ESR measurement of the irradiated frog leg bones [115].

Gruiz and Kiss [116] irradiated chicken to 4 kGy or 50 kGy at  $-12^{\circ}\text{C}$  to  $-18^{\circ}\text{C}$ . At 4 kGy the fatty acid profile of the irradiated tissues did not differ from that of the control, but the samples irradiated to 50 kGy had decreased stearic and palmitic acid contents and increased peroxide values. Oxidative changes were also measured by others [117], who irradiated fatty tissues of broiler chickens to 4 to 10 kGy under dry ice and then stored the samples at  $-18^{\circ}\text{C}$ . Peroxide values, conjugated diene, and carbonyl contents were used to demonstrate that oxidation increased with radiation doses and continued to increase during

a storage period of up to 3 months. The addition of tocopherol to the feed of chickens, while they were raised, increased the storage stability of the irradiated samples, but the authors concluded that there was a definite limit to the storage period during which irradiated chickens retained acceptable sensory properties.

Hansen *et al.* [118] also addressed the problem of sensory quality. They irradiated chicken to 3 to 12 kGy at 0–5°C and stored some samples irradiated to 3 kGy at 4°C for up to 14 days. C<sub>6</sub> to C<sub>9</sub> volatiles varied directly, and odor acceptability varied indirectly with dose. Chickens irradiated to 3 kGy had a better odor score after 7 days of storage than the unirradiated control or the irradiated chicken immediately after treatment. After 14 days of storage the irradiated chicken had an odor score similar to that of fresh chicken, but the odor of the unirradiated control was highly unacceptable (probably because of microbial growth).

A compound isolated from irradiated chicken but not found in raw or cooked chicken was identified as 2-dodecylcyclobutanone [119]. The compound persists in chicken after 18 days of storage and in chicken cooked before or after irradiation [120]. It has been suggested that the compound might serve as a marker for irradiated chicken (see below). Evidence was presented that the compound is formed from palmitic acid [121].

### 4.3 Marine Products

Much of the early research on the effect of  $\gamma$ -radiation on fish and other marine products was carried out by scientists at the Bureau of Commercial Fisheries Technological Laboratory at Gloucester, Massachusetts, and by the U.S. Army Natick Laboratories at Natick, Massachusetts. This work and that conducted by other laboratories until the late 1970s have been capably summarized by Diehl [104]. Because these aquatic species contain relatively high concentrations of long-chain fatty acids with multiple unsaturated bonds, irradiation was frequently carried out under the exclusion of air to minimize oxidative degradation. The volatile radiolytic compounds formed from the

marine muscle lipids approximated those expected from a consideration of the fatty acid composition. In addition, amines and sulfur-containing volatile compounds were also formed from irradiation of the muscle tissue.

Both of these types of compounds are present in fresh, unirradiated controls as well, but they are more prominent after storage of the controls and after irradiation of fresh samples.

More recent research has been concerned with the extension of shelf life of fish by reduction of the microbial burden by radiation processing. Low levels of  $\gamma$ -radiation are sufficient to accomplish this, but the processing and storage are best carried out at ice temperature and in packaging that excludes, or at least limits, air access. Indian mackerel were irradiated in ice to 1.5 kGy and stored at 0–2°C or at 10°C [122]. Storage at the lower temperature increased shelf life by 28 days over the unirradiated control, as judged by organoleptic values. At 10°C, shelf life was extended by only 15 days. During storage of Indian mackerel, an economically important species on that sub-continent, volatile fatty acids, mostly formic and acetic acid, are formed and cause loss of organoleptic acceptability [123]. Irradiation to 1.5 kGy retards the formation of the volatile acids and thereby extends shelf life. There is a close correlation between volatile fatty acids and organoleptic score.

As would be expected, susceptibility to radiation-induced oxidation is a function of the fat content of the fish. Irradiation to 1–2 kGy of fatty fish (herring) caused oxidative rancidity, but this was not a factor in the irradiation of semi-fatty fish (red perch) [124]. Irradiation of fresh, iced catfish fillets to 0.5 to 1.0 kGy reduced the microbial load significantly [95]. TBA values did not increase at 0.5 kGy compared to controls, but did increase after 1.0 kGy. Poole *et al.* [126] irradiated several species of fish and seafood to 1, 3, and 5.0 kGy in crushed ice. Processing to 1 kGy resulted in a 1.5 to 4.0 log reduction in bacterial load without a reduction in sensory quality. Higher doses decreased sensory values in some species.

Microbial contamination presents a serious problem in fish minces created with mechanical deboning devices. Minced fillets of cod were irradiated to 3 kGy at –20°C to reduce the

number of microorganisms [127]. The minces were evaluated for 3 months of frozen ( $-18^{\circ}\text{C}$ ) storage. The irradiated samples did not differ significantly from the unirradiated controls in a number of important parameters, such as texture, water holding capacity, lipid oxidation, lipid hydrolysis, and others.

It is interesting to note that oils extracted from fish are more prone to irradiation-induced oxidation than are the lipids contained in fish tissue. Several fish oils mixed with starch and irradiated to 1–4 kGy gave rise to considerable amounts of oxidation products [128]. Adam *et al.* [129] irradiated herring fillets to 50 kGy at  $0^{\circ}\text{C}$  in the absence of oxygen. They could not observe any changes in the fatty acid profile, including the amounts of eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), even after storage of the samples for 4 weeks at  $0^{\circ}\text{C}$ . On the other hand, when they extracted the oil from the fillets and irradiated the oil or oil/water emulsions, they observed significant destruction of both the 20:5 and the 22:6 acids. They theorized that proteins in the herring fillets protect polyunsaturated acids from radiolysis. They concluded that herring can be processed at the recommended dose (1–2 kGy) without the loss of nutritionally important components.

Dried anchovies were irradiated to 5 kGy and stored for 6 months at  $25^{\circ}\text{C}$  in nylon/polyethylene bags [130]. The small oxidative changes that were observed in the irradiated product did not diminish the quality of its odor or flavor.

#### 4.4 Plant Products

Early reports of studies concerning the irradiation of fruits and other plant-derived materials make little mention of radiolytic effects of the  $\gamma$ -radiation on lipids [104]. Most fruits and vegetables are fairly low in lipid content, and lipid oxidation is not particularly prominent at 1 kGy or less, the dose required to achieve insect disinfestation. More recent investigations applied much higher doses for experimental purposes and examined results with more sophisticated and sensitive instrumentation. Nevertheless, the observed changes in plant lipid composition due to radiolytic effects were often minor in nature and had

minimum impact on the perceptible qualities of the product. An additional complication of the experimental process is that the lipid composition of many plants changes considerably with the degree of ripeness. It is therefore necessary that the sample to be irradiated and its unirradiated control have identical stages of ripeness.

Bancher *et al.* [131–133] irradiated peanuts and walnuts to 5 kGy and to 100 kGy and analyzed lipid classes by thin-layer chromatography with densitometric quantitation. Irradiation of peanuts resulted in a decrease in triacylglycerols with corresponding increases in monoacyl- and diacylglycerols. Triacylglycerols containing hydroperoxy substituents were observed after irradiation at the higher dose. Walnuts also yielded considerable amounts of hydroperoxidized triacylglycerols and polymeric materials. Polar lipids of irradiated peanuts and walnuts suffered some degradation to phosphatidic acids and lyso compounds. Gas chromatographic analysis of the fatty acid spectrum of peanut and walnut lipids before and after irradiation showed that irradiation caused a relative decrease in the more highly unsaturated fatty acids and a corresponding increase in the saturated acids.

Others [134] irradiated peanuts to 2.5 and 20 kGy and stored the irradiated samples, as well as unirradiated controls, at  $-14^{\circ}\text{C}$  and at ambient temperatures. Irradiation caused little change in oil composition, except that linoleic and linolenic acids decreased somewhat in irradiated peanuts stored at ambient temperatures for 1 year. In the latter sample there were also slight increases in peroxide and TBA values and in conjugated diene. Almonds and cashew nuts were irradiated to 1.0, 1.5, and 2.0 kGy and to 1.0, 2.0, 3.0, and 4.0 kGy, respectively, and stored at  $5 \pm 2^{\circ}\text{C}$  for 6 months [135]. No change in free fatty acid content was observed. There was an increase in lipid peroxidation, but it was insufficient to be detected organoleptically.

Half-ripe mangoes were irradiated to 0.25 kGy [136], and mango and papaya pulp were irradiated to 0.75 kGy and strawberry pulp to 2.0 kGy [137] without detectable radiolytic changes in fatty acid composition.

Exposure of high-moisture (30.5%) soybean seeds to 60 kGy [138] and buckwheat seeds to 4.0 kGy [139] doses of ionizing radiation caused significant decreases in, but did not eliminate, lipoxygenase activity. The fatty acid composition was largely unaffected. On the other hand, the relative amounts of fatty acids of soybean protein products were observed to change after irradiation to 3.0 and 5.0 kGy [140].

Two potato cultivars were irradiated to 0.1 and to 1.0 kGy to inhibit sprouting and were stored subsequently for 1, 4, and 26 weeks at 5°C and at 20°C [141]. Irradiation decreased the crude lipid and the phospholipid content, and these decreases continued during storage, although less on storage at 5°C than at 20°C. After eight rice cultivars were irradiated to 5, 10, and 15 kGy [142], the linoleic acid content of the phospholipids decreased and their free fatty acid content increased correspondingly. The neutral lipids were stable to the radiation.

#### 4.5 Miscellaneous Foods

The potential benefits from the irradiation of eggs were recognized in the 1950s, an active period in the investigation of food irradiation. It was recognized then that irradiation to doses of 3–10 kGy, energy applications that were sufficient to achieve the desired reduction in microbial populations, often resulted in unsatisfactory odor or flavor of the food products in which the irradiated eggs were incorporated. Information on the chemical effects of the irradiation of eggs was rather limited at that time [104].

Some of the lacking information was supplied by a study of the chemical effects of  $\gamma$ -radiation on egg powders [143, 144]. Irradiation of whole egg powder and of egg yolk powder to 1 to 10 kGy at dose rates of 4, 0.4, or 0.04 Gy/s was carried out in the presence or absence of air. Triacylglycerols decreased and mono- and diacylglycerol increased with dose, but acidity and free fatty acids remained constant. Hydroperoxide formation also increased with dose in the presence of air, but not linearly. Hydroperoxide formation had an induction dose of about 2.5 kGy and varied inversely with dose rate. Carotenoid destruction

in the absence of air proceeded linearly with dose. The organoleptic properties of scrambled eggs and of mayonnaise prepared from irradiated products were indistinguishable from the unirradiated controls when samples were irradiated in air up to 3 kGy and in the absence of air up to 5 kGy.

Mass spectrometry provided evidence that the irradiation of pork fat to 30 kGy at  $-45^{\circ}\text{C}$  under vacuum leads to triacylglycerol recombination products such as triacylglycerol dimers, propanedioldiester/triacylglycerol adducts, and others [145]. Such compounds had previously been identified among the radiolysis products of model triacylglycerols (see above).

9-Oxononanoic acid was isolated from sunflower oil irradiated to 60 kGy and from lard irradiated to the same absorbed dose in a linear accelerator [146, 147]. This compound, the structure of which was confirmed by mass spectrometry, was not present in unirradiated lard but was detected, although in low concentration, in unirradiated sunflower oil.

#### 4.6 Sterols in Foods

The well-known susceptibility of cholesterol to oxidation with the formation of numerous oxidation products has been described above. The adverse human health implications of some of these products have led to an intensified search for such oxidation products in foods. As a result, it has been demonstrated that significant amounts of cholesterol oxides can occur in a large variety of foods of animal origin [148–151], particularly in powdered dairy products [152, 153], processed marine foods [154, 155], and muscle foods [156, 157]. Harsh processing conditions and prolonged storage time seem to aggravate the problem [158–161]. Formation of cholesterol oxides during deep frying operations in which animal fats are used has been a special concern [162–164].

Despite the demonstration in model systems that exposure of cholesterol to ionizing radiation gives rise to cholesterol oxidation products, few irradiated foods have been examined to determine whether cholesterol in foods is oxidized similarly.

Spray-dried egg powder irradiated to 1 to 6 kGy [165] was shown to contain the cholesterol 5,6-epoxides, as well as the 7-hydroxycholesterols and 7-ketocholesterol. The same cholesterol oxides were prominent products of the irradiation of raw meats [166–167]. 6-Ketocholestanol, a product not formed in the autoxidation of cholesterol, was measured at levels below 1 ppm in chicken that had been irradiated to 10 kGy at 0–4°C [168].

The plant sterol content of unrefined vegetable oils has been reported to range from about 60 mg/100 g for palm oil to about 900 mg/100 g for corn oil [169–171]. These amounts are considerably higher than the cholesterol content of most meat and poultry, which usually averages between 75 and 85 mg/100 g. Refining reduces the phytosterol content of the oils considerably, perhaps by as much as 30% [172–174].

The principal sterol component of most vegetable fats and oils is  $\beta$ -sitosterol, a compound that has been reported to form oxidation products that are structurally similar to those of the cholesterol oxides [175]. These have been detected and measured in some food products [162, 176]. Very little is known about the intestinal absorption and health effects of phytosterol oxides, and very little research has been reported on their manner of formation or their transfer from vegetable oils to processed foods. This lack of knowledge is of some concern, because conditions during the frying of foods (high temperature in the presence of air) are ideal for the formation of these oxides. The level of concern is increased because of the structural similarities between phytosterol and cholesterol oxides and the known toxicity of some of the latter. It has been shown that during simulated frying experiments, as much as 25% of the sterol content of the frying medium was “lost” [177, 178]. The effect of ionizing radiation on food phytosterols has not been reported.

## 5. IRRADIATION EFFECTS ON ODOR AND FLAVOR

High-dose irradiation of meats at ambient temperatures, intended to sterilize these products, gives rise to off odors and off flavors that are unacceptable [3, 20]. These adverse sensory

quality attributes can be minimized by changes in the treatment parameters. Low-dose irradiation at temperatures well below freezing and at reduced pressures or under modified atmospheres are treatment conditions that reduce off odors.

Merritt *et al.* [179] reported that the irradiation odor and flavor of beef increase with dose and decrease with temperature. These authors obtained excellent correlation between off odor and off flavor and between off flavor and total volatiles (hydrocarbons, carbonyls, and sulfur compounds). Hydrocarbons and carbonyls are derived mostly from the radiolysis of lipids. The implication is, then, that a significant part of the off flavor comes from the lipid component of meats.

On the other hand, Sudarmadji and Urbain [180] irradiated various animal muscle tissues at 5–15°C to varying doses from 0.1 to 50 kGy in sealed bags and heated the bags in boiling water for 30 min. A taste panel then evaluated the flavor of the cooked tissues to determine the threshold doses at which off flavors became noticeable. There was no apparent relationship between fat content and irradiation flavor. Among the threshold doses recorded were turkey, 1.75 kGy; beef and chicken, 2.5 kGy; and lamb, 6.25 kGy.

The sensory threshold value of 2.5 kGy has been confirmed by others [181, 182]. Shay and co-workers [181] note that irradiation-induced oxidative changes present a particular problem with fatty tissues. They suggest that a balance must be struck between the desired improvement in microbiological quality and the magnitude of induced organoleptic changes when conditions for radiation processing of a particular food are selected. Hanis *et al.* [182] add that although irradiation at temperatures as low as –40°C can avoid detectable organoleptic changes, such low temperatures are not always technologically feasible and, at any rate, add substantial additional cost to the process.

The threshold value of 2.5 seems to have some importance in the irradiation of beef. When fresh top round of beef was irradiated to 2 kGy at 22°C, trained panels did not detect off odors, even after extended storage at 1°C [183]. Shelf life, as

judged by microbial counts, was extended by 17 days compared to nonirradiated samples.

The origin of the objectionable odor that develops when meat is irradiated has not yet been fully determined. For beef, at least, there is some agreement [20, 104] that the typical “wet dog odor” often associated with irradiated beef is different from the odor generated when isolated beef fat is irradiated.

A combination of low-dose (1 kGy) irradiation and controlled atmosphere was reported to be successful [184, 185] in reducing the objectionable odors of irradiated fresh pork. Thorough purging with nitrogen gas was also successful in reducing adverse organoleptic changes in irradiated milk [186], but microbial contamination under anaerobic conditions was considerably higher than in the presence of air.

Milk and other dairy products have long been known to be particularly susceptible to changes in odor and flavor resulting from irradiation [23, 68], and these changes have been reported to increase, in the case of milk, with fat content [20]. An attempt to sterilize milk by irradiation in sealed cans to 45 kGy at  $-80^{\circ}$  or at  $-185^{\circ}\text{C}$  resulted in a product that had an extremely bitter flavor [187].

## **6. IDENTIFICATION OF IRRADIATED FOODS**

During the past decade radiation processing of foods has made great strides in moving from the research stage into commercial practice and has become more common in international trade. This progress has increased the need for accurate, reliable, and sensitive analytical methods capable of distinguishing between nonirradiated and unirradiated foods.

Such methods are needed to ensure that national regulations governing radiation processing are observed, to confirm compliance with regulations governing labeling of irradiated and unirradiated foods, to increase consumer confidence in irradiated products, to protect against multiple irradiations, to control international trade of irradiated foods, and to provide information regarding the absorbed dose.

An ideal method would meet the following requirements:

1. The measured response is specific for the irradiation process and is not caused by other processing techniques, or by storage conditions.
2. The method is accurate, reliable, reproducible, rapid, and inexpensive.
3. It is easy to perform, away from sophisticated laboratories, on small amounts of food.
4. It is applicable over a wide range of doses and has detection limits below the minimum dose likely to be applied to a specific food.
5. The method permits estimation of the absorbed radiation dose.
6. It can be used on a wide range of foods.

No method with such a range of attributes has yet been developed, nor has any such method appeared on the horizon. A number of methods applicable to different specific foods or groups of foods, however, are in an advanced state of development. Several of these have been tested in interlaboratory trials. Such procedures must be properly validated so that they can serve to support legal proceedings. Especially important in the validation process have been the collaborative international studies sponsored by the European Union's Community Bureau of Reference (BCR) and by the International Atomic Energy Agency (IAEA) under its worldwide effort on Analytical Detection Methods in Irradiation Treatments (ADMIT).

There have been several recent and comprehensive reviews describing advances in the development of diagnostic tests [188–194]. Details of the test procedures can be found in these reviews and the references quoted therein. For the purposes of this chapter a summary of the most prominent methods will suffice.

## **6.1 Long-Chain Hydrocarbons**

Nawar and Balboni reported in 1970 [195] that hydrocarbons result from the radiolytic conversion of fatty acids in foods, that the amounts of hydrocarbons produced increase with increasing dose and temperature of irradiation, and that this chemi-

cal reaction could be used as a diagnostic tool for the detection of irradiated food. The hydrocarbons are formed by the decarboxylation of fatty acids, and those of greatest diagnostic value have one or more double bonds. The original procedure involved extraction of the fats, concentration of the volatiles by vacuum distillation, and measurement of the individual components by GC and/or GC/MS. This method is still frequently referred to as the "Nawar method," although modifications in the procedure have been made by others. Thus a Florisil column [114, 115, 196], an alumina column [197], or liquid chromatography (LC) [198] have been used in the concentration step, and LC-LC has been employed to achieve further purification [198]. In all of these modifications, however, the final quantitation of the hydrocarbon products employs GC or GC/MS.

The Nawar method has been studied intensively in international collaborative trials sponsored by the European Union (BCR) and by the International Atomic Energy Agency (ADMIT). This is probably the developmentally most advanced of the fat-based methods. It has been reported to have a detection limit of less than 0.15 kGy when applied to irradiated sunflower, olive, or peanut oils and has also been used to detect irradiated avocado pears and irradiated poultry meat [199]. It was employed to distinguish between irradiated and unirradiated nutmeg [200].

## 6.2 2-Alkylcyclobutanones

Compounds of this type were first isolated after high-dose irradiation of triacylglycerols, and a mechanism for their formation from fatty acids was proposed [26]. More recently, 2-dodecylcyclobutanone was detected in irradiated (4.7 kGy) chicken, even after 20 days of storage, but not in raw or cooked nonirradiated samples [119]. The cyclic ketone was reported to be derived from palmitic acid in irradiated chicken [121]. The amount of 2-dodecylcyclobutanone increases linearly with dose at 1–10 kGy [120], and this linearity of dose response was even found in radiation-sterilized chicken irradiated to 10–60 kGy at  $-40^{\circ}\text{C}$  and then stored at  $-46^{\circ}\text{C}$  for 12 years [201]. A test

procedure, based on the formation of 2-alkylcyclobutanones and intended for the diagnostic detection of irradiated poultry and meat, has been the subject of collaborative international studies.

An example of the use of a combination of such test methods to identify irradiated foods in international trade was reported recently [202]. Application of the hydrocarbon method and the 2-alkylcyclobutanone method clearly distinguished between irradiated and unirradiated whole egg and egg products. Analysis of the cellulosic packaging materials by electron spin resonance spectroscopy (see below) confirmed the results of the two chemical methods.

### **6.3 Electron Spin Resonance Spectroscopy**

Although ESR-based detection methods are not capable normally of identifying irradiated, lipid derived materials, they are included here to provide the reader with a brief summary of the utility of this important physical procedure.

Irradiation of foods containing bone or other crystalline material such as the shells of mollusks and crustacea results in the generation of free radicals that produce characteristic ESR signals [203]. The intensity of the signal varies linearly with dose [204–206], and the method has frequently been reported to be suitable for distinction between irradiated and unirradiated foods [e.g., 203, 206–209]. Much of the investigative effort has been applied to an examination of the ESR signal induced in irradiated chicken bones. It has been suggested that this signal comes from hydroxyapatite [209], that bones from dissimilar carcass sites give different signal intensities that correlate with bone crystallinity [210], and that the lower limit of ESR detection is 50 Gy [204, 211]. The signal is stable over extended periods of storage at reduced temperatures [203, 205, 209, 210, 212–214]. An estimation of the original dose of irradiated foods can be obtained by re-irradiation of the sample to a known dose and extrapolating the ESR signal to zero dose [203, 211, 215, 216]. In interlaboratory trials [217, 218] correct distinctions were made between irradiated and unirradiated chicken, and estimations of the original dose were acceptable.

#### 6.4 Miscellaneous Diagnostic Methods

A few additional methods, of the several that have been proposed for the identification of irradiated foods, are discussed briefly here to illustrate the considerable scope of the activity that has taken place in this field in recent years. This activity is believed to be indicative of a growing interest worldwide in the potential benefits of irradiation as a food processing technique.

Luminescence techniques are based on the principle that energy absorbed by some types of foods during irradiation is stored and can be released later, upon the application of a stimulus, in the form of light. Both chemiluminescence and thermoluminescence (TL) have been tested, but the latter has been shown to be the more reliable. A wide variety of irradiated herbs, spices, seasonings, vegetables and fruit can be distinguished from unirradiated samples by means of TL. The origin of the TL signals is primarily minute amounts of mineral debris present in the food [218]. It has been demonstrated that the sensitivity and reproducibility of the signal can be enhanced by appropriate extraction techniques that concentrate the inorganic impurities [218]. TL has been modified and expanded by others [219, 220]. Collaborative studies have demonstrated that TL is accurate and reliable, more so than chemiluminescence [221]. A recent report described the experimental details of the TL technique [222].

Radiation damage to starch, pectin, and cellulose, major macromolecular components of some spices and dried vegetables, results in changes in viscosities of aqueous suspensions of these materials [223, 224]. For some specific foods it has been possible to differentiate between irradiated and unirradiated samples, but the method requires more research to determine, for example, why some viscosities increase whereas others decrease after irradiation.

The primary objective of food irradiation is, of course, to reduce the population of microflora. It has been suggested [225] that the reduction in viable microorganisms due to irradiation can be estimated by comparing the aerobic plate count (APC) with a count obtained using the Direct Epifluorescent Filter

Technique (DEFT). In the samples examined, the DEFT count determined the number of microorganisms before irradiation. Hence the difference between the DEFT count and the APC is equal to the number of organisms rendered nonviable by irradiation. It has been pointed out that heat treatment would produce an effect similar to that of radiation in this case, and that this method could not be applied to irradiation-sterilized meat that has been subjected to heat inactivation of enzymes before irradiation.

Another method proposed for the use of microorganisms is one in which the production of volatile acids and volatile bases was measured in unirradiated and irradiated beef, chicken, mutton, and pork after inoculation with *Aeromonas hydrophila*. Both acids and bases decreased with increasing dose [226].

Chicken, beef, and pork irradiated and stored at  $-20^{\circ}\text{C}$  retained carbon monoxide, which was measured later by gas chromatography [227]. The amount of CO increased with dose and did not decrease materially after storage of more than a year at  $-20^{\circ}\text{C}$ .

## 7. SUMMARY

After more than 40 years of intensive study, treatment with ionizing radiation has become increasingly important as a safe food processing procedure. In its application to meat, poultry, and marine food products its principal purpose is to increase shelf life by the reduction of spoilage and pathogenic microorganisms in these foods.

The effect of radiation treatment on the lipids in foods has been studied in detail both in model systems and in the foods themselves. Under practical processing conditions the upper dose level for most foods is 10 kGy or less, sometimes much less, before deterioration of odor and flavor renders the food unacceptable. Because of the chemical complexity and compositional variability of most foods, the chemical effect of such low doses on food lipids is minute and often barely distinguishable from the effects of natural processes such as autoxidation.

Nevertheless, significant progress has been made in understanding the radiation chemistry of lipids and in identifying the various types of radiolysis products that can be formed, if only in the barest of traces. This understanding has led to sophisticated diagnostic methods capable of detecting irradiated food based on the radiolysis of lipids. These methods are now being validated by radiation scientists in international collaboration. Once proved reliable, these methods will add immeasurably to the regulation and control of national and international trade in irradiated foods.

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