X-Ray Irradiation as a Microbial Intervention Strategy for Food

Scott R. Moosekian,[1] Sanghyup Jeong,[2] Bradley P. Marks,[1,2] and Elliot T. Ryser[1]

[1]Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824; email: mooseki1@anr.msu.edu, ryser@msu.edu

[2]Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, Michigan 48824; email: jeongsa1@msu.edu, marksbp@msu.edu

Annu. Rev. Food Sci. Technol. 2012. 3:493–510

First published online as a Review in Advance on December 9, 2011

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

This article's doi:
10.1146/annurev-food-022811-101306

1941-1413/12/0410-0493$20.00

Keywords

produce, seafood, pathogen inactivation, dose measurement, process control and validation, consumer acceptance

Abstract

First recognized in 1895, X-ray irradiation soon became a breakthrough diagnostic tool for the dental and medical professions. However, the food industry remained slow to adopt X-ray irradiation as a means for controlling insects and microbial contaminants in food, instead using gamma and electron beam (E-beam) irradiation. However, the reinvention of X-ray machines with increased efficiency, combined with recent developments in legislation and engineering, is now allowing X-ray to actively compete with gamma irradiation and E-beam as a microbial reduction strategy for foods. This review summarizes the historical developments of X-rays and discusses the key technological advances over the past two decades that now have led to the development of several different X-ray irradiators capable of enhancing the safety and shelf life of many heat-sensitive products, including lettuce, spinach, tomatoes, and raw almonds, all of which have been linked to high profile outbreaks of foodborne illness.

INTRODUCTION

Production of safe food is of paramount importance for the well-being of society. Despite ongoing efforts, foodborne illness remains a vexing challenge. In the United States, increasing numbers of foodborne illness cases are being traced to consumption of fresh produce, nuts, and other dry food products, with three large multistate outbreaks of *Escherichia coli* from fresh-cut baby spinach and lettuce documented in 2006. Most recently, cantaloupe was implicated in the second largest outbreak of foodborne listeriosis in the United States. These outbreaks pose an ongoing threat to public health because of the surge in sales of ready-to-eat fresh-cut produce; national and international distribution patterns; and the lack of a definitive kill step to ensure the elimination of microbial pathogens during culling, washing, and further processing. In light of these events, ionizing radiation is attracting renewed attention as a potential nonthermal microbial intervention strategy to ensure the safety of fresh fruits and vegetables. One of the newest strategies involves the development of machines that generate low-energy X-ray electromagnetic waves for in-line processing, with these units likely to receive greater consumer acceptance than gamma irradiators that rely on a radioactive source.

Electromagnetic waves are inherent to the world in which we live, with human exposure coming from many diverse sources, ranging from the sun to cellular phones. Typical electromagnetic wavelengths/frequencies vary in size from 10^2 m (AM radio signals) to 10^{-10} m (X-rays). Based on the duality of matter as hypothesized by de Broglie in 1925 (Khan 2003), electromagnetic waves also can be referred to as particle radiation. Depending on the wavelength/frequency, electromagnetic waves will yield different effects, with the particles or photons from shorter wavelengths able to more easily penetrate solid materials. Radiation—defined as the process by which electromagnetic waves or particles pass through a medium—is classified as either ionizing or nonionizing. If the electromagnetic wave energy is greater than the minimum required for ionization of atoms, it is categorized as ionizing radiation and if not it is nonionizing radiation. Ionizing radiation includes both direct (electrons, protons, alpha particles, heavy ions) and indirect radiation (X-ray, gamma rays) (Podgoršak 2006).

Based on particle mass and speed, varying amounts of energy are transmitted to a food product or any other material upon impact. The amount of energy deposited in a unit mass (J kg^{-1}) is measured using a standard unit called a gray (Gy), which is named in honor of the British physicist Louis Harold Gray, the father of modern-day radiobiology. Typical ionizing radiation doses for treating food products range from 1 to 44 kGy. The dose required to reduce a microbial population by 90% (i.e., 1 log) is termed the D_{10} value (kGy) (Molins 2001). Another convenient energy unit in atomic and nuclear physics is the electron volt (eV), which is the kinetic energy, equal to 1.602×10^{-19} J, needed to accelerate an electron across a one volt electric potential difference (Khan 2003). The energy level for gamma rays is approximately 1×10^6 eV or (1 MeV), with X-ray being in the range of a few keV to MeV.

Radioactive materials and particle accelerators are the two main sources for ionizing radiation. Gamma rays are a natural by-product from the decay of radioisotopes (e.g., ^{60}Co or ^{137}Cs) and are emitted in all directions, whereas electron beams (E-beams) generated from particle accelerators can be aimed at specific targets. Consequently, each type of radiation has its inherent advantages and disadvantages in food, medical, and other types of applications.

HISTORY OF X-RAY

Ionizing irradiation—including gamma ray, E-beam, and X-ray—has long been recognized as a viable cold pasteurization strategy for reducing the levels of both pathogenic and spoilage microorganisms in a wide range of foods for the purpose of enhancing food safety and product shelf life. The introduction of X-rays as a source of ionizing irradiation dates back to the late nineteenth century, when German physicist W. C. Roentgen first observed the generation of radiation during his experiments with Hittorf-Crookes tubes, also known as modified cathode ray tubes. In his 1895 paper, "Eine neue Art von Strahlen" ("A New Kind of Ray"), Roentgen described how rays exiting the device generated transparencies of several objects, including paper, tinfoil, wood, rubber, and flesh. Roentgen noted that "if one holds a hand between the discharge apparatus and the screen, one sees the darker shadow of the bones within the slightly fainter shadow image of the hand itself" (Roentgen 1895). Roentgen termed the unknown light rays that were emitted from the tube X-rays, forming the basis behind the function of medical X-rays.

Further understanding of X-rays and their properties as a source of ionizing irradiation developed in accordance with the discovery of radioactive elements. In 1896, A. H. Becquerel observed a form of radiation similar to that of Roentgen, stemming from elemental uranium, finding that when a photographic plate was exposed to salts of the element, a distinct impression was left on the plate (Strutt 1904). Marie and Pierre Curie expanded upon the work of Becquerel with experimentation into understanding the source of energy and radioactivity of elements. Marie Curie further clarified the radiation emitted from radioactive materials, describing the similarities between Roentgen's rays and alpha, beta, and gamma rays, as penetrating rays that are unaffected by a magnetic field (Curie 1961).

X-RAYS AS A FORM OF IONIZING IRRADIATION

X-rays, or Roentgen rays, appear next to gamma rays in the electromagnetic spectrum at frequencies of 10^{16} to 10^{19} Hz. The somewhat lower energy photons emitted by X-rays are formed from the interaction of a charged particle with matter, either from replacing displaced electrons from a low-lying orbit or through bremsstrahlung, also known as braking radiation (Newton 1963). Machine sources of X-rays primarily use bremsstrahlung, where active photons emitted when high-velocity electrons strike a dense metal target, such as tungsten, tantalum, or gold, are directed toward the desired object. These high-energy particles alone may also generate a lower level of ionization, the technology of which has been used in the development of high-energy E-beams.

Research into the ionizing effects of X-ray radiation did not begin until several years after its initial discovery, when researchers began to explore the power and effect of X-rays on various targets. At this time, researchers were still relatively unaware of the potential mechanism of action. Much of the work with X-rays during the early 1900s focused on food preservation and the control of insects or pests that negatively impact the quality of food and other consumer goods.

Through the early part of the twentieth century, it was generally concluded that the technology and efficiency of X-rays was simply too expensive for large-scale industrial use. In 1912, W. D. Hunter documented some of the first effects of Roentgen rays on insect control. In his review, he concluded that for the insects being tested with the technology of the time, there were no indications of any practical applications of X-rays for the destruction of insect pests (Hunter 1912). A few years later, Morgan & Runner (1913) detailed the use of Roentgen rays in controlling the tobacco or cigarette beetle's infestation of tobacco products. Their work coincided with an attempt by the American Tobacco Company, under the assistance of Hunter, to commercialize X-rays for this particular purpose. The commercial design used two seven-inch X-ray tubes operating at 64,000 to 70,000 volts, with a current of 1.5 to 2.5 mA passing through the tubes. However, this treatment was ineffective in sterilizing tobacco because of variable X-ray penetration.

Using a subsequently modified Roentgen ray that allowed the operator to control both the intensity and penetrating power, higher doses applied at 600 mA (15 mA $\times$ 40 min) to infected tobacco yielded more promising results, showing signs of beetle egg infertility (Runner 1916). The American Tobacco Company later implemented an in-line conveyor system for irradiating boxes of cigars in 1929, using a water-cooled X-ray at a maximum power of 30 mA at 200 kV. However, the equipment still proved to be unsuitable for continuous use, as too much time and energy were wasted in machine maintenance and operation (Diehl 1995).

In 1918, a U.S. patent was granted to D. C. Gillett for his design of an "Apparatus for Preserving Organic Materials by the Use of X-Rays" (Gillett 1918). Gillett's design was intended to "destroy utterly any destructive insects or other animal life that would tend to destroy perishable articles, or to sterilize these insects and prevent the further propagation of their species." In 1921, based on a design similar to Gillett's, the U.S. Department of Agriculture began research through the Zoological Division of the Bureau of Animal Industry on X-ray-initiated inactivation of *Trichinella*, the parasite in pork responsible for human trichinosis. The end results, however, were inconclusive and did not justify X-ray as a feasible means of trichinae destruction. X-rays appeared to injure the organism and/or disrupt reproduction, but the trichinae exhibited considerable variation in susceptibility (Schwartz 1921).

The impact of X-rays and other forms of ionizing irradiation on matter had a monumental impact in the United States throughout the 1940s and 1950s. During the 1940s, interest focused on the application of irradiation toward preserving foods in an effort to extend the food supply. In 1953, President Eisenhower proposed the Atoms for Peace Program in a speech to the United Nations, describing and advocating the use of ionizing irradiation of food as a means to decrease world hunger by limiting the need for preservation and reducing pests. However, as the United States entered the Cold War, interest shifted toward the negative effects of ionizing irradiation on the nutritional properties of food and the side effects of consuming irradiated foods.

Breakthroughs in the actual mechanism of X-rays and ionizing irradiation on cells and microorganisms took place toward the middle of the past century in conjunction with developments in DNA research. During that time, Bergonie & Tribondeau (1959) reported that X-rays were more effective against rapidly growing cells, noting that they destroyed tumors as opposed to the surrounding tissue. Further information on the historical developments of X-rays and ionizing irradiation can be found in reviews by Josephson (1983) and Whitmore (1995), and greater detail pertaining to research done by the U.S. Army can be found in reviews by Josephson et al. (1978) and Brynjolfsson (1979).

MECHANISM OF ACTION

When an atom is exposed to X-rays, energy transactions occur between the projected photons and the orbiting electrons. These interactions result in a net transfer of energy from X-rays to electrons in the absorbing material, raising the electron excitation level (Newton 1963). Excitation, resulting from a low level of energy, moves an electron further out in its atomic orbit, thereby increasing the net energy. Ionization then occurs when the energy level sufficiently increases to produce highly reactive positive and negative ions by the removal of an orbiting electron (Wilkinson & Gould 1998).

The extent to which ionization occurs in irradiated matter depends on the energy of the photon and the physical properties of the matter. Activity generated by the photons of X-rays may result in one of two energy absorption processes in the irradiated material, compton scattering or photoelectric absorption (Miller 2005). When using low-energy photons, photoelectric absorption is generally seen with all of the photon's energy transferred to the electron, which then goes on to interact with other atoms. Compton scattering occurs at higher energy levels where photon interactions are confined to outer, loosely bound electrons, causing only a portion of the photon energy to be absorbed by the encountered electron (Pizzarello & Witcofski 1975). Under these conditions, the initial photon and any excited or ionized particles may continue to react. It is estimated that an electron may produce 30,000–40,000 additional ionization processes and 45,000–80,000 excitations (Nawar 1986).

The impact of charged particles on matter is classified according to the type of contact. Ionized or excited molecules may exert their effect by either direct contact of the photon with their target, i.e., the direct effect, or by the formation of cations created from target components such as water, i.e., the indirect effect (Podgoršak 2006). Direct effects occur randomly and are dependent upon the electron density of the biological material. Water, a major component of foods and biological material, is the primary target for energy coming from the X-ray source. Reactions with photons and water lead to the generation of highly reactive free hydrogen and hydroxide radicals that are split from the hydrogen bond. Excited water molecules can further react to produce hydrogen and hydrogen peroxide, the only stable end products of water radiolysis (Miller 2005).

The effects of ionizing irradiation can be seen throughout each component of an organism; however, the primary target remains the DNA. Approximately 20% of this attack is on DNA sugars and 80% on bases, with thymine being the most sensitive, followed by cytosine, adenine, and guanine (Moseley 1990). Pollard (1966) attributed this observation to the relative mass of DNA compared with the organelles of the cell, stating that radiation sensitivity of organic substances is proportional to their molecular weight. He estimated that a dose of 0.1 kGy would damage 0.005% of the amino acids, 0.14% of the enzymes, and 2.8% of the DNA within a given cell. Within DNA, most strand breaks occur from the scission of the C-3′ phosphate ester bond, producing 5′-PO_4 and 3′-PO_4 termini in a 3:1 ratio (Johnston & Stevenson 1990). Both single- and double-strand breaks can occur in DNA based on the random action of ionized particles with the latter occurring far less frequently.

DOSE MEASUREMENTS

Radiation doses are measured using ionizing radiation-sensitive materials that can be classified according to their accuracy and range. Based on accuracy of the measurement, the following four categories are now recognized: (*a*) primary standards (∼1% to 2% uncertainty) maintained by national standards laboratories, (*b*) reference standards (∼3% uncertainty) for calibrating radiation environments and routine dosimeters, (*c*) transfer standards for establishing traceability of an irradiation facility, and (*d*) routine standards (∼5% to 10% uncertainty) for radiation process quality control, absorbed-dose monitoring, and mapping (ISO/ASTM 2005). A water calorimeter (∼10^{-5} to 10^4 Gy) or ionization chamber is used for primary standards, an alanine dosimeter (∼10^{-1} to 10^4 Gy) is used for reference standards, and a clear PMMA (polymethylmethacrylate) or radiochromic film (∼10^2 to 10^5 Gy) is used for routine dosimeters (Molins 2001). Of these, alanine-EPR (electron paramagnetic resonance) and radiochromic dosimeters are the most popular. The alanine-EPR dosimetry system uses an alanine dosimeter (film or pill type) and EPR spectroscopy to measure free radicals (ISO/ASTM 2004a, Maltar-Strmecki & Rakvin 2004). In radiochromic dosimetry (film type), a spectrophotometer is used to measure radiation-induced color changes on a film as a series of absorption bands (ISO/ASTM 2004b, Mehta & Parker 2011). Alanine dosimeters are stable over long periods of time, whereas calibration curves must be continually established for radiochromic films.

THE NEED FOR ALTERNATIVE MICROBIAL REDUCTION STRATEGIES

Across the globe, interest in ionizing irradiation has increased steadily since the beginning of the millennium, with the market for irradiation equipment increasing from 19 billion to over 25 billion

Table 1 Approved uses for food irradiation (USFDA 2010)

Use	Year	Dose
Control of insects in wheat and flour	1963	= 0.5 kGy
Inhibiting spouting in potatoes	1964	= 0.15 kGy
Pork carcasses for *Trichinella spiralis*	1986	= 1 kGy
Culinary herbs, seeds, spices, vegetable seasonings	1986	= 30 kGy
Delay ripening of fruit and disinfesting fruits and vegetables of insects	1986	= 1.0 kGy
Fresh or frozen, uncooked poultry products	1990	= 3 kGy
Frozen, packaged meats used solely in NASA space flight programs	1995	Minimum dose 44 kGy
Refrigerated or frozen, uncooked meat products	1997	= 4.5 kGy (refrigerated) = 7.0 kGy (frozen)
Fresh shell eggs for *Salmonella*	2000	= 3.0 kGy
Seeds for sprouting	2000	= 8.0 kGy
Fresh or frozen molluscan shellfish for *Vibrio* bacteria	2006	= 5.5 kGy
Iceberg lettuce and spinach	2008	= 4.0 kGy

U.S. dollars. The United States alone claims roughly one quarter of this spending (Parker 2005). Worldwide, various irradiation technologies are now being used in at least 55 countries to treat food products (IAEA 2009). Renewed interest in ionizing irradiation has developed in response to continued outbreaks traced to fresh produce, including lettuce (Ethelberg et al. 2010, Irvine et al. 2009, Nygard et al. 2008, Sodha et al. 2011), spinach (Grant et al. 2008, Wendel et al. 2009), and raw nuts (Danyluk et al. 2007, Isaacs et al. 2005, Kirk et al. 2004) because these products are are adversely affected by thermal processing. From 1998 to 2007, a total of 1,999 outbreaks and 35,554 illnesses were associated with consuming meat, poultry, and seafood, with 684 outbreaks and 26,735 cases of illness from produce (CSPI 2009).

Microbial reduction strategies for fresh fruits and vegetables have remained largely ineffective because of current growing/harvest/processing practices and the nature of the material. Contamination of leafy greens and other produce, as discussed by Doyle & Erickson (2008), can occur in the field from irrigation water and animals as well as during processing from contaminated flume water. Because many produce items are traditionally consumed raw, to offer some degree of protection to the consumer, processing of fresh-cut produce typically includes one or more washing steps using chemical sanitizers. However, these means are largely ineffective, as evidenced from recent outbreaks, given that bacterial levels are typically reduced only 1–2 logs on the product (Sapers 2001).

U.S. Food and Drug Administration's approval of irradiation began in 1963, with doses of up to 0.5 kGy being allowed for controlling insect infestation of wheat and flour (**Table 1**). This list has since expanded with 12 approved uses for irradiation, the most recent addition coming in response to the 2006 outbreaks that were traced to *Escherichia coli* O157:H7 on spinach and lettuce. In a report issued by the joint FAO/IAEA/WHO study group on the nutritional and safety impact of food irradiation, foods irradiated at doses below 10 kGy were deemed to be wholesome (WHO 1997).

X-RAY AS A VIABLE ALTERNATIVE

Until very recently, food irradiation as a microbial reduction strategy focused almost exclusively on gamma rays and E-beams, with gamma rays identified as the only energy efficient means for cold pasteurization. This large body of literature on food irradiation has been reviewed elsewhere with respect to meat and poultry (O'Bryan et al. 2008), fish and seafood (Arvanitoyannis et al. 2009a, Venugopal et al. 1999), and fruits and vegetables (Arvanitoyannis et al. 2009b). It is only within the past decade that X-ray irradiation has garnered some attention as a viable microbial reduction strategy based on its now proven efficacy, minimal environmental impact, and potential for direct installation in commercial processing lines. Given the extensive shielding and other hazards associated with the radioactive sources for gamma radiation (e.g., ^{60}Co or ^{137}Cs), X-ray and E-beam are becoming more practical alternatives. In addition, these nonradionuclide machine-source irradiators can be turned on and off by a switch, allowing for more efficient commercial processing and greater operator control.

ADVANCES IN X-RAY TECHNOLOGY

Reinvention of X-ray machines with increased efficiency, combined with recent developments in legislation and engineering, is now allowing X-ray to actively compete with gamma irradiation and E-beam as a microbial reduction strategy for foods. In the generation of bremsstrahlung, one of the unfortunate outcomes is the inadequate conversion of energy from integrated photons to integrated electrons, which decreases process efficiency. This has been viewed by some as the primary limitation to X-ray use for commercial applications, but is also an area of debate. Initially, the approved maximum energy level permitted for X-rays was set at 5.0 MeV; however, at an October 16–18, 1995 meeting of the FAO/IAEC/WHO in Vienna, Austria, it was concluded that X-ray machines producing up to 7.5 MeV "can be used without any concern about induced radioactivity but would be a satisfactory, efficient and cost effective addition to other radiation sources available for food processing" (ICGFI 1995). In light of this conclusion, FDA amended their food additive regulations in December 2004 by establishing a new maximum permitted energy level for X-rays of 7.5 MeV, provided that the X-rays are generated from machine sources that use tantalum or gold as the target material (Federal Register 2004).

The effective increase in energy from 5.0 to 7.5 MeV is not substantial, yet it is still important, as the emission efficiency increases proportionately with an increase in electron energy, as indicated in **Table 2**. Using Monte Carlo simulation, Meissner et al. (2000) found that bremsstrahlung yield—a measure of the emission efficiency—doubled when the energy level increased from 5.0 to 10.0 MeV. They also estimated a greater degree of penetration when an equivalent uniform dose was applied. Consequently, more powerful X-ray machines for food irradiation are now being designed based on these new rules. One manufacturer (L3 Communications Titan Pulse Sciences Division, San Leandro, CA) is preparing to develop an irradiator that incorporates

Table 2 Properties of X-ray at energy levels of 10.0, 7.5, and 5.0 MeV (Meissner et al. 2000)

Energy (MeV)	Emission efficiency (%)[a]	Double-sided treatment ($g\ cm^{-2}$)[b]	Dose uniformity ratio (D_{max}/D_{min})[c]
10	16.2	43	1.54
7.5	13.3	38	1.54
5.0	8.23	34	1.54

[a]Efficiency of the bremsstrahlung yield (X-ray generation) in the forward direction assuming normal electron incidence on to tantalum converter (conversion efficiency).
[b]Aerial density ($g\ cm^{-2}$) of stacked polyethylene plates (density 0.96 $g\ cm^{-3}$, dimension 49 × 80 × 40 cm height).
[c]Max to min dose ratio for the stacked polyethylene plates.

both a 7.5 MeV electron linear accelerator capable of generating 100 kW of average power and a tantalum converter.

In 2009, these L3 devices were used as an 18 kW E-beam linear accelerator and a 15 kW, 10 MeV X-ray linear accelerator, and then compared with a hydrostatic pressure treatment for inactivation of *E. coli* O157:H7 in ground beef inoculated at 10^3 CFU g^{-1} (Schilling et al. 2009). Using X-ray and E-beam doses of 2 kGy, the *E. coli* O157:H7 population decreased below the limit of detection, whereas hydrostatic pressure (300 mPa) did not completely eliminate the pathogen. Furthermore, sensory panelists found the irradiated and nonirradiated control samples to be comparable and overall more acceptable than ground beef treated with hydrostatic pressure. No potentially hazardous volatile compounds were detected in irradiated or hydrostatic pressure-treated samples.

Further technological advancements in X-ray technology have focused on improved efficiency. Rad Source Technologies (Suwanee, GA) led these developments with their RS 2000 X-ray design. This machine uses a patented Rad Source RAD+ Chamber and a point source X-ray tube (Rad Source Technologies 2008) to deliver a uniform irradiation dose over a large area. Additional energy is provided from a reflector of low Z (atomic number), high-density material that is positioned within the chamber to reflect radiation back to the product (Gueorguiev 2002).

The RS 2000 X-ray irradiator has been tested at several institutions across the United States, with the bulk of this work having been done at Mississippi State University. Using this irradiator at a maximum dose of 2.0 kGy (dose rate of 1 kGy per 50 min at 145 kV and 19 mA), Robertson et al. (2006) were able to reduce initial *Listeria monocytogenes* populations of 4.4 log CFU g^{-1} in vacuum-packaged smoked mullet to undetectable levels with no recovery seen when the fillets were held for 90 or 17 days at 3°C or 10°C, respectively. Sensory panelists were also unable to detect any differences between the treated and untreated samples (Robertson et al. 2006). However, the 100 min exposure time to achieve these results was cited as being very problematic for the industry.

In 2009, Collins et al. (2009) expanded on this research by evaluating the same system and operating conditions in treating fresh channel catfish fillets for the reduction of *L. monocytogenes*, mesophilic aerobic bacteria, psychrotrophic bacteria, and total coliforms. Their findings were similar to those of Robertson et al. (2006), with 40%, 27%, 0%, and 7% of samples yielding *L. monocytogenes* after receiving irradiation doses of 0, 0.5, 1.0, and 1.5 kGy, respectively. They also reported the development of an off aroma in unirradiated control samples during storage at 5°C over 17 days, which was not detected on the irradiated fillets, with these changes attributed to fewer inherent bacteria and spoilage organisms on the treated fillets.

In other developments, Rad Source Technologies received a U.S. patent (number 7,346,147) for 4pi X-ray emitters (Kirk & Gorzen 2008). The manufacturer claims that this device delivers higher dose rates, comparable to gamma irradiators, through the use of an extended anode design in which X-rays are generated from a cylindrical rather than a point source, using all of the photons produced (Rad Source Technologies 2008). Their design results in both the creation of X-rays proceeding through the anode along its length and X-rays that are reflected back through the length of the anode and throughout the circumference of the cylindrical anode (Kirk & Gorzen 2008).

This 4pi technology has been used by Rad Source (RS) technology in the construction of their RS 2400 and RS 2500 irradiators. Specifications for the RS 2400 X-ray irradiator in **Table 3** illustrate how high dose rates can be achieved under low-energy conditions (<1 MeV). However, the operating capabilities of the cabinet remain a limitation to the design, allowing only small batches of product to be treated within an exposure chamber measuring 91.4 × 60.0 × 63.5 cm.

Efficacy of this improved RS 2400 irradiator (dose rate of 1.0 kGy per 16 min at 145 kV and 45 mA) has been demonstrated through a series of reports on the treatment of shellfish. In 2009, whole live and half-shell oysters were irradiated to inactivate *Vibrio parahaemolyticus* (Mahmoud

Table 3 Characteristics of the Rad Source 2400 Irradiator (Mehta & Parker 2011)

Characteristic	Value
Maximum tube voltage	150 kV
Maximum tube current	45 mA
Maximum power	6.75 kW
X-ray converter	12 ìm gold
Dose rate (to water) in center of rice-filled canister	14.1 Gy min^{-1}
Dose energy ratio at this location	0.0374 Gy $(kW\ s)^{-1}$

& Burrage 2009) and later *Vibrio vulnificus* (Mahmoud 2009a). In this work, *V. parahaemolyticus* populations decreased more than 6 logs to levels below the limit of detection using X-ray irradiation doses of 0.75, 2.0, and 5.0 kGy for pure-culture, half-shell, and whole-shell oysters, respectively. Under the same conditions, *V. vulnificus* was more susceptible, with reductions of greater than 6 logs seen after exposing the pure-culture, half-shell, and whole-shell oysters to doses of 0.75, 1.0, and 3.0 kGy, respectively. In addition, the shelf life of whole oysters could be extended using a dose of 5.0 kGy, as evidenced by oyster survival at the high dose, and inherent microorganisms were reduced to levels below the limit of detection. Mahmoud (2009b) reported equally effective results using the same system on ready-to-eat shrimp, demonstrating more than a 6 log CFU reduction for *E. coli* O157: H7, *Salmonella* Enterica, *Shigella flexneri*, and *V. parahaemolyticus* using X-ray doses of 2.0, 4.0, 3.0, and 3.0 kGy X-ray, respectively. Lower X-ray doses of 0.75 kGy also significantly reduced the initial microflora in ready-to-eat shrimp samples from 3.8 $\pm$ 0.2 to $<$1.0 log CFU g^{-1}.

Mahmoud later went on to demonstrate the efficacy of this same X-ray irradiator for a series of produce items, including spinach (Mahmoud et al. 2010), iceberg lettuce (Mahmoud 2010a), and Roma tomatoes (Mahmoud 2010b). In these experiments, results indicated a greater than 5 log reduction for *E. coli* O157:H7, *L. monocytogenes*, *S.* Enterica, and *S. flexneri* at 2.0, 1.0, and 1.5 kGy for iceberg lettuce, spinach, and Roma tomatoes, respectively. In addition, treatment at these dose levels significantly decreased inherent microflora, which in turn enhanced product shelf life during refrigerated storage. Results obtained on leafy greens were similar to those reported for both gamma (Niemira et al. 2002, Niemira 2008) and E-beam (Gomes et al. 2008) radiation (**Table 4**).

The RS 2400 irradiator was also evaluated for inactivation of *Enterobacter sakazakii* in milk (Mahmoud 2009c). X-ray doses of 5.0 and 6.0 kGy reduced ($P < 0.05$) *E. sakazakii* populations to $<$1 log CFU ml^{-1} in skim milk and milk containing $\geq$1% fat, respectively. However, this study did not investigate the possible negative impact of X-ray doses on product quality and rancidity, which is an important consideration in high-fat products.

Rayfresh Foods (Ann Arbor, MI) also developed a patent-pending process termed The Rainbow Process, which is based on low-energy ($<$1 MeV) X-rays (Rayfresh Foods 2009). This technology has been evaluated at Michigan State University over the past five years using a wide range of products, including lettuce, spinach, parsley, asparagus, blueberries, almonds, walnuts, chestnuts, and ground beef. Research to date on this system has been conducted using a pilot-scale irradiator (maximum power 4 kW, operating energy 70 kV) comparable in design to the RS 2400 irradiator. Rayfresh Foods currently is focused on development of a commercial-scale irradiator for in-line processing.

Experiments assessing the efficacy of this new technology against *E. coli* O157:H7 in ground beef yielded a D_{10} value of 100 Gy (Jeong et al. 2007), which was significantly lower than previously published D_{10} values for gamma or E-beam irradiation ($\sim$270 Gy) (Thayer & Boyd 1993). This system also has proven to be highly effective against *E. coli* O157:H7 on leafy green vegetables.

Table 4 Microbial efficacy (D_{10} value) of X-ray irradiation on various products

Products	Microorganisms	Inoculation method	D_{10} value (kGy)	Reference	X-ray irradiator
Oysters (whole live and half shell)	*Vibrio parahaemolyticus*	Immersion	2.0 log reduction kGy^{-1} (whole shell)[a]	(Mahmoud & Burrage 2009)	150 kV 6.75 kW^{-1} (RS 2400, Rad Source Technologies Inc., Alphretta, GA)
			4.9 log reduction kGy^{-1} (half shell)[a]		
Trypticase soy broth (TSB)	*Enterobacter sakazakii*	Mix	0.41 ± 0.1	(Mahmoud 2009b)	
Skim milk			0.54 ± 0.04		
Low-fat milk (1%)			0.65 ± 0.02		
Low-fat milk (2%)			0.71 ± 0.3		
Whole-fat milk (3.5%)			0.74 ± 0.03		
Spinach (leaves)	*Escherichia coli* O157:H7	Spot	1.1	(Mahmoud et al. 2010)	
	Listeria monocytogenes		1.0		
	Salmonella Enterica		1.2		
	Shigella flexneri		0.96		
Iceberg lettuce (shredded)	*E. coli* O157:H7	Spot	4.4 log reduction kGy^{-1}[(a)]	(Mahmoud 2010a)	
	L. monocytogenes		4.1 log reduction kGy^{-1}[(a)]		
	S. Enterica		4.8 log reduction kGy^{-1}[(a)]		
	S. flexneri		4.4 log reduction kGy^{-1}[(a)]		
Roma tomatoes (whole)	*E. coli* O157:H7	Spot	0.39 ± 0.5	(Mahmoud 2010b)	
	L. monocytogenes		0.66 ± 0.1		
	S. Enterica		0.56 ± 0.1		
	S. flexneri		0.98 ± 0.3		
Shrimp (frozen, cooked, peeled ready to eat)	*E. coli* O157:H7	Immersion	1.1 ± 0.01	(Mahmoud 2009a)	
	S. Enterica		1.3 ± 0.03		
	S. flexneri		1.2 ± 0.01		
Mullet (vacuum-packaged smoked)	*L. monocytogenes*	Immersion	1.6 log reduction kGy^{-1}[(a)]	(Robertson et al. 2006)	145 kV 2.76 kW^{-1} (RS 2,000, Rad Source Technologies, Boca Raton, FL)
Iceberg lettuce (leaves)	*E. coli* O157:H7	Immersion	0.040 ± 0.001	(Jeong et al. 2010b)	70 kV 4 kW^{-1} (Rayfresh Foods Inc., Ann Arbor, MI)
		Spot	0.078 ± 0.008		

[a]D_{10} values were not reported.

Treatment of iceberg lettuce yielded a D_{10} value of 0.040 kGy (Jeong et al. 2010), which is 3.4 times lower than the previously reported value of 0.136 kGy using gamma radiation (Niemira et al. 2002) (**Table 4**). When ten stacked leaves were irradiated from both sides, a dose of 0.2 kGy was achieved at the center of the stack, with a surface dose of 1 kGy corresponding to a 5-log reduction for *E. coli* O157:H7 at the center of the stack. Lower D_{10} values were also observed when spinach (0.035 kGy), parsley leaves (0.0522 kGy), and parsley stems (0.067 kGy) were surface inoculated with *E. coli* O157:H7 (Moosekian et al. 2010a).

Efficacy against *Salmonella* on tree nuts (almonds and walnuts) has also been demonstrated (Jeong et al. 2012). Nuts were inoculated with *Salmonella* Enteritidis PT30 or *Salmonella* Tennessee, and conditioned to different water activities (0.23–0.84). The efficacy was significantly ($P < 0.05$) affected by both nut type and water activity, with greater inactivation on the surface of almonds than on walnuts, for equivalent doses, and maximum resistance at a water activity ~0.6. Also, irradiation of uninoculated samples to a dose that would achieve a 5-log reduction (1.1 and 2.4 kGy for almonds and walnuts, respectively) resulted in no perceivable sensory changes in almond quality, as determined by a consumer panel, but did result in a decrease in acceptability for the walnuts, indicating the importance of product-specific data in evaluating the technology.

The Palletron system is another proposed commercial design for implementing X-ray technology in food-processing facilities. This irradiator increases dose uniformity through a rotational pallet system comprising a radiation source, adjustable collimator, turntable, and control system (Kotler & Borsa 2003), with the goal of reducing the dose uniformity ratio (DUR) or the ratio between the maximum and minimum dose absorbed by different areas of the food or within a food container (Grandison 2006). According to Lazurik et al. (2007), application of X-ray beams from multiple angles and orientations increases both dose uniformity and efficiency. When applied from four sides, beam efficiency was more than 60%, which allowed large objects to be processed at a DUR < 1 (Lazurik et al. 2007). Monte Carlo simulation studies with the Palletron further demonstrated that it is possible to reach a DUR > 1.5 for all densities up to 0.8 g cm^{-3} while preserving a high treatment capacity (Stichelbaut et al. 2004a).

HURDLE APPROACH FOR PATHOGEN CONTROL

Combining irradiation with other treatments, including chemical preservatives and growth inhibitors in a hurdle approach, has been proposed as an additional option for enhancing product safety and quality. Thayer et al. (2006) found that irradiation and chlorination acted synergistically in the inactivation of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* on fresh produce. In a separate report, Foley et al. (2004) determined that although water, chlorine (200 ppm), and irradiation (1.05 kGy) significantly reduced levels of *E. coli* O157:H7 on cilantro, combined use of irradiation with a wash treatment was superior to irradiation alone.

When integrating an X-ray irradiator as a final kill step into any established processing line for leafy greens, X-ray exposure of the packaged product likely would be preceded by one or more washing steps that typically involve the use of chemical sanitizers. In other recent work, Moosekian & Ryser (2010b, 2011) reported variable efficacy of X-ray irradiation against *E. coli* O157:H7 on baby spinach, based on prior sanitizer exposure during flume washing. Whereas one commercial chlorine-based sanitizer afforded protection against irradiation, a synergistic effect was observed between the treatments using a peroxide-based sanitizer.

X-RAY IRRADIATION PROCESS CONTROL AND VALIDATION

Irradiation doses need to be uniformly delivered to the product to avoid an overdose because the energy from X-ray (unlike E-beam) is attenuated exponentially along the projection axis of the material. Therefore, processing models are needed to predict both X-ray dose distribution in the food matrix and microbial inactivation efficacy. Because of the complexities in generating ionizing radiation, transport, and interaction with matter, construction of a purely analytical model is clearly challenging. Unlike mono-energetic gamma rays and E-beam, X-ray's multi-energetic energy spectrum results in angular dependence of the radiation, energy dependence of X-ray absorption, and product particle scattering (Miller 2003).

For food irradiation, the dose estimation methods and modeling techniques resemble the radiation treatment plans for medical patients (Ay & Zaidi 2005, Borsa et al. 2002, Reynaert et al. 2007). Given recent computer advances, simulations of particle transport and interactions are now being used to estimate dose distributions within the intended target. A typical stochastic process suited for this type of simulation is the Monte Carlo method, in which the history of each particle (trajectory, interactions) is traced in detail (Cantone & Hoeschen 2011). A detailed comparison among several Monte Carlo particle transport codes, including MCNPX, GEANT4, FLUKA, MARS, and PHITS, is available (Los Alamos National Laboratory 2011). Typically, the composition of the food matrix is mapped using computer tomography images (CT scan), after which a three-dimensional image of the object is reconstructed with embedded density/composition information (Borsa et al. 2002). This material composition model is then coupled with Monte Carlo particle transport codes to predict the radiation dose received at a specific location (kGy), with this dose then converted into a process lethality based on the D_{10} value for the target organism.

Some readily available Monte Carlo codes include MCNPX (McKinney 2011) (license needed from the Radiation Safety Information Computation Center, Oak Ridge National Laboratory, Oak Ridge, TN) and GEANT4 (Agostinelli et al. 2003) (free and downloadable from **http://geant4.web.cern.ch/geant4/index.shtml**). The main difference is that GEANT4 can handle electromagnetic field problems, which enables the modeling of accelerated electrons in an electromagnetic field.

Using these Monte Carlo codes, Stichelbaut et al. (2004a, 2004b) successfully modeled the Rhodotron® TT300 (X-ray from 5, 7, and 10 MeV) and Palletron™ using the GEANT Monte Carlo simulation toolkit. For complex foods, including chicken carcass/broccoli (Kim et al. 2007, Kim et al. 2006), apple (Brescia et al. 2003, Kim et al. 2006), an apple surrogate (Rivadeneira et al. 2007), and bagged spinach (Gomes et al. 2008), this modeling procedure was successful using machine source irradiation. Even though Monte Carlo simulation provides an accurate dose-distribution map of the product, this is not an ordinary task. Therefore, Miller (2003) developed a faster and more convenient analytical/empirical model for X-ray irradiation that can be validated using integrated TIGER series Monte Carlo codes. After model validation, an economic analysis is then needed to assess industry feasibility. Based on available economic models, all three available irradiation technologies, gamma, E-beam, and X-ray, have their own benefits and hindrances that must be specifically addressed by each user (Kunstadt 2001, Sadat 2004).

CONSUMER ACCEPTANCE

The efficacy and safety of food irradiation have been recognized for more than a century, especially in recent decades, by the scientific community as well as numerous health organizations and governmental agencies. Given this consensus, food irradiation is now approved in more than 50 countries, 30 of which are irradiating multiple commodities (Mostafavi et al. 2010). However, some hurdles to further expansion of food irradiation remain because of negative consumer perception of irradiated foods, industry costs associated with adopting the technology, and the labeling requirement (e.g., Radura symbol) by regulatory agencies.

Despite increased efficacy and continued research on the safety of irradiated foods, some consumers remain skeptical (Lyndhurst 2009). Therefore, the issues are how much negative perception can be tolerated and can consumer perceptions of food irradiation be improved through education? Consumer acceptance, perception, and attitude are influenced by many factors including socio-demographic/economic status, risk-benefit perceptions, knowledge, trust in the source of information, and labeling (Rollin et al. 2011). In the United States (Bruhn 2001), Europe (Rollin et al. 2011), Brazil (Behrens et al. 2009, Martins et al. 2008), Chile (Junqueira-Goncalves et al. 2011), South Korea (Byun et al. 2009), Argentina (Curzio & Croci 1998), Africa (Mostafavi et al. 2010), Turkey (Gunes & Tekin 2006), and Egypt (El-Fouly et al. 2002), consumer attitudes and negative perceptions (or fear) of food irradiation improved after consumer education. Commodity-specific consumer acceptance of various irradiated products, including ground beef (Lorenzen & Heymann 2003, Spaulding et al. 2006, Wheeler et al. 1999), fruits (Deliza et al. 2010, Martins et al. 2008), pork (Fox et al. 2002, Wolfe et al. 2005), fish (Aworh et al. 2002), meat (Eustice & Bruhn 2010), onions (Curzio & Croci 1998), apple cider (Yulianti et al. 2004), and turkey meat (Lee et al. 2003), also has been assessed. Although most of this work focused on consumer acceptance, adoptive behavior of irradiation by retailers was also assessed (Jaenicke et al. 2006). A recent trend has been seen toward improved consumer acceptance of certain health-promoting food additives, such as antioxidants, which can also reduce radiation by-products and increase product value (Over et al. 2010, Yan et al. 2006). One approach to improving consumer acceptance involved the public display and advertisement of irradiated foods (Furuta 2004). After consumer acceptance improves to the level of other technologies, food irradiation is likely to become far more widely adopted by the food industry.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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