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Irradiation of Fresh and Minimally Processed Fruits, Vegetables, and Juices*

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INTRODUCTION

Vegetable products, including fresh produce, pre-cut salad vegetables, and fresh juices, typically receive only minimal processing. Often eaten raw or only lightly cooked, these commodities play an important dietary role in many societies.

* Mention of brand or firm name does not constitute an endorsement by the U. S. Department of Agriculture above others of a similar nature not mentioned.

Although concerns related to the presence of pathogenic bacteria on food products have historically been associated with animal products such as meat, milk, and eggs, vegetable products have come under international investigation as sources of food-borne illness. This increased prevalence is due to changes in farm technology, processing and shipping practices, and changing consumption patterns associated with minimally processed fruits, vegetables, and juices (Hedberg et al., 1994; Tauxe et al., 1997; NACMCF, 1999). Beuchat (1996) discussed the incidence of human pathogens on fresh produce, citing contact with contaminated soil, water, compost, harvesting or processing equipment, or human handlers as means by which produce may become contaminated.

Application of conventional preservation and antimicrobial tools, such as heat or chemical wash treatments, to the special needs of produce is being widely investigated (Hotchkiss and Banco, 1992; Beuchat, 1998); among the alternative antimicrobial technologies is the use of ionizing radiation. Irradiation as a food treatment is endorsed by a variety of professional and governmental organizations such as the United States Food and Drug Administration (Anonymous, 2000b), U.S. General Accounting Office (Anonymous, 2000a), American Dietetic Association (Wood and Bruhn, 1999), and the U.N. World Health Organization (WHO, 1999).

The majority of food irradiation research efforts in recent decades have been devoted to treatment of meat products, and irradiation as a terminal control step is becoming more widespread for a variety of meat products in the U.S. (see Sommers, chap. 14, this volume). Low-dose irradiation may be implemented during postpackaging as a terminal control step and is therefore of interest to producers of nonthermally pasteurized (NTP) juices, fresh sprouts, pre-cut vegetables, prepared salad mixes, fruit salads, and other minimally processed vegetable products (NFPA, 2000), but applications are as yet limited.

Vegetables were among the first experimental subjects in studies of physiological response to irradiation (Guilleminot, 1908; Miege and Coupe, 1914). Produce, fruit juices, milk, eggs, and meat were all subjects of research to extend shelf-life with irradiation (Proctor et al., 1955; Thayer et al., 1996). Initial studies with irradiated produce typically used relatively high doses and were intended to achieve pasteurization-level reductions in spoilage bacteria and fungi (Diehl, 1995). Although this research helped to define the effective doses to eliminate spoilage organisms, the doses employed often exceeded the maximum radiation tolerances of vegetable commodities tested, resulting in loss of quality (Howard and Buescher, 1989; Prakash et al., 2000b). For this reason, irradiation was generally regarded as unsuitable for application to produce (Maxie and Abdel-Kader, 1966; Yu et al., 1996; Osterholm and Potter, 1997). However, more recent research with lower radiation doses, i.e., less than 3 kiloGray (10 kGy = 1 Mrad), has suggested a role for irradiation as one of several "hurdles" in fruit and vegetable processing (Tauxe et al., 1997; Thayer and Rajkowski, 1999; Anonymous, 2000a).

Preservation of fresh produce is limited to refrigeration and judicious use of modified atmosphere packaging (Sumner and Peters, 1997). Unlike meat, fresh produce is living tissue, able to conduct respiration, maintain water relations with its environment, and continue synthesis of defense compounds and secondary metabolites (Salisbury and Ross, 1984). Irradiation can alter these processes, leading to

changes in firmness, aroma, color, or taste (Yu et al., 1996; Prakash et al., 2000b). Fresh and lightly processed vegetable products, as well as enzymatically active NTP juices, are relatively sensitive to storage conditions of temperature, humidity, light levels, and packaging atmosphere. The effects of irradiation on plant physiology and surface-associated microbial ecology are expressed during refrigerated handling and storage (Howard and Buescher, 1989; Al-Kahtani et al., 2000; Prakash et al., 2000a).

The purpose of this chapter is to discuss low-dose ionizing irradiation of minimally processed fruit, vegetable, and juice products to increase food safety. The discussion will draw on current research to describe pathogen prevalence and survival in various commodities; the applicability of the various irradiation technologies and their interaction with other antimicrobial measures; and factors that can influence pathogen sensitivity to radiation and postirradiation product quality. As cut vegetables, prepared salads, and other minimally processed vegetable products increase in popularity and global distribution, application of antimicrobial measures, including irradiation, will serve to protect the public from the threat of food-borne illness.

MICROFLORA OF MINIMALLY PROCESSED VEGETABLE PRODUCTS

Plant parts such as leaves, stems, fruits, and roots typically support 10^3 to 10^6 colony forming units (CFUs) per gram of plant tissue (Sumner and Peters, 1997; Mercier and Lindow, 2000). The vast majority of these microorganisms consist of nonpathogenic bacteria that interact with the plant and with each other, often forming biofilms as part of the phytoplane ecology (Carmichael et al., 1999; Fett, 2000). These organisms come from the environment in which the plants are grown, including the soil, water, air, and manure and compost, as well as from postharvest handling, processing, and shipping (Beuchat, 1996). Formation of bacterial biofilms on the inert surfaces of processing equipment such as steel, glass, rubber, and plastics is a well-known phenomenon (Costerton et al., 1995), but the widespread nature of the biofilm habitat on leaf and root surfaces has been illustrated only in recent years (Fett, 2000).

The biofilm habitat is a complex community of many bacterial species bound to the plant surface in a durable exopolysaccharide matrix. Many of the details regarding this communal existence are still being defined, but it is known that the sheltered matrix environment allows for interspecies exchange of nutrients and metabolites and protects the resident bacteria from desiccation and exposure to antimicrobial chemical rinses (Morris et al., 1997; Fett, 2000). The microbial populations on and in fruits and vegetables are carried into derivative juices or fresh-cut products during processing (Parish and Higgins, 1988; Sizer and Balasubramaniam, 1999). Fresh-cut produce cannot be heat pasteurized without loss of quality. Although the majority of fruit and vegetable juices sold in the U.S. are pasteurized and therefore present little risk of food-borne pathogens, fresh NTP juices command a premium price for flavor and aroma. NTP juices also have a relatively short shelf-life due to their microbial load (Buchanan et al., 1998).

The increasing significance of human pathogens on fresh fruits, vegetables, and juices has been recognized in recent years (Beuchat, 1996; Tauxe et al., 1997; Thayer and Rajkowski, 1999). Like nonpathogens, pathogenic organisms come from the

environment in which the plants are grown as well as from postharvest handling, processing, and shipping (Beuchat, 1996). Because of repeated international outbreaks of salmonellosis and enterohemorrhagic *Escherichia coli* O157:H7 infections associated with raw sprouts (Taormina et al., 1999), in 1999 the U.S. Food and Drug Administration warned the public against consumption of this commodity. *Salmonella* and *E. coli* O157:H7 contamination of NTP juices (Cook et al., 1998; Buchanan et al., 1998) and contamination of widely consumed products such as lettuce, tomatoes, cabbage, and melons have led to an increased effort to address the problem of bacterial contamination of minimally processed fruits, vegetables, and juices.

IRRADIATION TECHNOLOGIES AND REGULATIONS

Diehl (1995) and Sommers (chap. 14, this volume) discuss the technologies used for irradiation of food products in detail. The advantages and disadvantages of the three commercial types of ionizing radiation are summarized in Table 13.1. It is important to note that each type of radiation ultimately leads to the introduction of high-energy electrons within the cells of the commodity and the resident microorganisms. Products are typically irradiated from two or more sides to ensure even coverage and maximum penetration. Ozone and heat are generated during the irradiation process for each of the technologies; proper ventilation and temperature control are therefore especially important for the irradiation of produce to avoid product degradation from these secondary effects (Maxie and Abdel-Kader, 1966).

Direct comparisons of the effects of various technologies on fruits, vegetables, or juice products are lacking, but in direct-comparison studies using meats, electron beam irradiation (1.25 or 3.0 kGy) was found to produce sensory and color changes in pepperoni very similar to those produced by thermal treatment (Johnson et al., 2000). However, while electron beam and gamma irradiation (1.5 or 3.0 kGy) were effective at eliminating *Salmonella typhimurium* from refrigerated beef steaks, electron beam was not as effective in the elimination of *Pseudomonas fluorescens*, a common spoilage bacterium found on meats and produce (Chung et al., 2000). Additional direct comparisons of the various irradiation methods are therefore warranted, including studies using fruit and vegetable products as the test subjects.

Under current U.S. regulations, dry or dehydrated vegetable-derived spices, seasonings, and flavorings as well as coloring agents (e.g., paprika) may be irradiated to 30.0 kGy (Anonymous, 2000b). The regulatory limit for fresh fruits and vegetables, however, is 1.0 kGy; furthermore, radiation is limited to the specific uses of disinfestation (i.e., inactivation of arthropod pests) and inhibition of produce growth and maturation (Anonymous, 2000b). In contrast, irradiation to control food-borne pathogens is permitted at higher doses for shell eggs (3.0 kGy), fresh and frozen poultry (3.0 kGy), fresh meats (4.5 kGy), and frozen meats (7.0 kGy). As of 2001, European regulators have approved only dry spices for irradiation (Gil-Robles and Funke, 1999).

In 1999, a coalition of U.S. food processors petitioned the U.S. Food and Drug Administration to amend U.S. regulations to allow doses of up to 4.5 kGy for a wide variety of refrigerated and ready-to-eat meat and vegetable products, including juices, with doses up to 10.0 kGy sought for frozen meat, vegetable, and juice

TABLE 13.1
Technologies for the Production of Ionizing Radiation

Factors	Electron Beam	X-ray	Gamma
Source	Electrons are generated on an emission coil and accelerated to high energy, 5 to 10 MeV.[a] Extensive cooling equipment required.	Created when high-energy electrons (up to 5 MeV) strike a metal plate (e.g., tungsten or tantalum alloys), typical conversion efficiency is 5 to 10%. Extensive cooling equipment required.	Radioactive decay of ^{60}cobalt (2.5 MeV) or ^{137}cesium (0.51 MeV). Cooling equipment required.
Mechanism	High-energy electrons cleave water molecules, creating oxygen and hydroxyl radicals that damage DNA, membranes. Direct cleavage of DNA also occurs.	High-energy photons stimulate atoms within target to release high-energy electrons, which cleave water molecules into radicals. Direct cleavage of DNA also occurs.	High-energy photons stimulate atoms within target to release high-energy electrons, which cleave water molecules into radicals. Direct cleavage of DNA also occurs.
Shielding required	During operation, >2 m concrete or <1 m steel/iron/lead. When source is powered off, no radiation is emitted.	During operation, >2 m concrete or <1 m steel/iron/lead. When source is powered off, no radiation is emitted.	>5 m water or >2 m concrete or <1 m steel/iron/lead. Source cannot be turned off, shielding of source must be the default position.
Speed[b]	Seconds	Seconds	Minutes (depending on source strength)
Penetrability[c]	6 to 8 cm, suitable for relatively thin or low density products	30 to 40 cm, suitable for all products	30 to 40 cm, suitable for all products

[a] MeV = million electron volts.
[b] Speed of dose delivery. The desired dose will vary, depending on the target organism and commodity irradiated.
[c] Penetrability in food, avg. density approximately 1 g/cm^3. This figure will vary for individual commodities due to localized variation in density associated with bone, voids, fibrous matter, etc.

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products. Elimination of human pathogens is the primary goal of these requested dose limits; the potential for extension of shelf-life is regarded as a secondary goal (Anonymous, 2000c; NFPA, 2000). Therefore, as scientific data accumulate and commercial interest in irradiation increases, U.S. and international regulations concerning the irradiation of fresh produce and juices are expected to change to reflect increased understanding (Anonymous, 2000c; Gil-Robels and Funke, 1999).

IRRADIATION PHYSIOLOGY AND MICROBIAL ECOLOGY

The physiological effects of ionizing radiation on spoilage organisms and human pathogens have been recently reviewed (Monk et al., 1994; Diehl, 1995). Cells are damaged when high-energy electrons fracture molecules, typically the water molecules, into highly reactive radicals, which can damage DNA, proteins, and cell membranes. Bacterial DNA is generally more sensitive to damage from radicals during active replication or transcription (Snyder and Poland, 1995). Plasmid DNA may also be damaged, disrupting and inactivating plasmid-encoded genes; pathogenic bacteria such as *Yersinia enterocolitica*, which carry plasmid-encoded virulence genes, may therefore have a reduced virulence after irradiation (Sommers and Bhaduri, 2001). Under conditions of very limited free water, such as on dried or frozen products, fewer hydroxyl and oxygen radicals are produced, and their mobility is reduced; direct breakage of DNA therefore becomes the predominant means of radiation damage (Diehl, 1995). Because the DNA molecule represents a relatively small target for direct interaction with the high-energy electrons, radiation resistance of bacteria on dried or frozen products increases (Snyder and Poland, 1995; Thayer and Boyd, 1995; Nieto-Sandoval et al., 2000).

Fungi are typically more resistant to radiation than are bacteria (O'Connor and Mitchell, 1991; Monk et al., 1994). D_{10} values, i.e., the amount of radiation necessary to effect a 90% (1-log) reduction, have been reported for yeasts and molds in the range of 1 to 3 kGy (Lovell and Flick, 1966; Lescano et al., 1991; Narvaiz et al., 1992), as opposed to D_{10} of 0.3 to 0.7 kGy for pathogenic bacteria on produce and juices (Buchanan et al., 1998; Rajkowski and Thayer, 2000; Niemira et al., 2001). Human pathogenic viruses are typically more resistant to radiation than bacteria or fungi. Due to relatively high D_{10} for pathogenic viruses such as hepatitis and Norwalk (Ostetholm and Potter, 1997), the doses required to achieve meaningful virus population reductions are typically in excess of what most fresh produce products can withstand without unacceptable softening and loss in quality (Howard and Buescher, 1989; Monk et al., 1994; Yu et al., 1996). Although low-dose irradiation has been shown to suppress some plant pathogenic fungi responsible for storage losses (Thayer and Rajkowski, 1999), irradiation as a primary control step in a pathogen reduction program is best suited to control of bacterial pathogens rather than viruses or fungal pathogens.

Comparatively few studies have been conducted on the effect of low radiation doses on the microbial ecology of indigenous microflora with respect to inoculated pathogens. Loss of rare individuals within the community, effect on species present in low densities, or other undefined changes in community structure and function

are typical concerns when considering the impact of antimicrobial measures (A. Matos, personal communication). A concern expressed by various authors (Thayer and Boyd, 2000) regarding irradiated foods is possible diminution of interspecies competition among the microorganisms; a suppression of the indigenous microflora by irradiation could lead to increased pathogen growth in storage.

Studies of irradiated meats have tended to support the position that, while irradiation of a product reduces the total population of bacteria on the sample, it does not lead to increased recolonization by inoculated pathogens (Licciardello et al., 1970; Tiwari and Maxcy, 1972; Thayer and Boyd, 2000). However, Matches and Liston (1968) found that *Salmonella* grew more rapidly on irradiated vs. nonirradiated fish fillets. Carlin et al. (1996) showed that the growth of *Listeria monocytogenes* inoculated onto previously disinfected leaves of endive (*Cichorium endiva*) was significantly enhanced, resulting in approximately 1.5 log units more pathogen on disinfected leaves vs. water-washed leaves within 4 days of storage at 10°C. This study relied on chemical disinfection rather than irradiation; however, the result indicates that the response of competitive phytoplane microorganisms to irradiation will be an important factor in establishing the appropriate use of this technology.

IRRADIATION OF FRUITS AND VEGETABLES

The Case for Irradiation Processing

With increasing consumption of fresh produce in the U.S. and increased globalization of the fresh produce market has come increasing concern over produce-associated food-borne illness (Beuchat, 1996; Tauxe et al, 1997; Taormina et al., 1999; Thayer and Rajkowski, 1999). Conventional antimicrobial procedures such as washing, chemical sanitization, thermal treatment, and modified atmosphere packaging have historically been developed and refined to suppress spoilage organisms effectively (Luh, 1997). Much research is dedicated to improving their efficacy against pathogenic bacteria such as *L. monocytogenes*, *Salmonella*, and *E. coli* (Beuchat, 1998; Jacxsens et al., 1999; Liao and Sapers, 2000).

It was long believed that pathogenic bacteria were not likely to be present in the interior of fruits and vegetables and that antimicrobial measures could reasonably be limited to surface disinfection (Anonymous, 1999). However, it is increasingly recognized that leaves, fruits, and seeds provide bacteria with numerous mechanisms to avoid these antimicrobial measures. As more recent research has shown, bacteria not only are likely to enter fruits and vegetables through natural openings (stomata, calyx, stem, stem scar, etc.), abiotic wounds, or phytopathogenic penetrations, but they also can survive within the produce for days or weeks (Anonymous, 1999; Buchanan et al., 1999; Fisher and Dolden, 1998; Takeuchi and Frank, 2000; Riordan et al., 2000). The consequences of internalization are reduction in the effectiveness of chemical treatments such as hot water, trisodium phosphate, chlorine, chlorine dioxide, and peroxyacetic acid (Zhuang and Beuchat, 1996; Beuchat, 1998; Pao and Davis, 1999; Fett, 2000).

The protective nature of bacterial biofilms also reduces the efficacy of antimicrobial measures (Morris et al., 1997; Fett, 2000). Biofilms can differ in size and

species composition, depending on the host plant species, the position of the leaf within the plant canopy, or their location on an individual leaf due to variations in substrate availability or microclimate suitability (Mercier and Lindow, 2000; Fett, 2000). Human pathogens such as *E. coli* and *Salmonella* are known to form durable biofilms on industrial surfaces (Dewanti and Wong, 1995; Korber et al., 1997). The participation of enteric bacteria, as well as the psychrotrophs *L. monocytogenes* and *Y. enterocolitica*, in pre-existing phytoplane biofilms formed by nonpathogenic bacteria is not known (Fett, 2000; Liao and Sapers, 2000). By penetrating sheltered areas in the surface, subsurface, or interior of fruits and vegetables, ionizing radiation inactivates bacteria throughout the leaf.

Microbiology of Irradiated Produce

Ionizing radiation extends shelf-life by inactivating storage pathogens (Thomas, 1983; Willemot et al., 1996). The use of irradiation to reduce human pathogens on produce, rather than on meat, poultry, or shellfish, is a comparatively new area of study (Monk et al., 1994; Thayer and Rajkowski, 1999). Most studies to date have shown that irradiation of produce typically reduces resident microorganisms as effectively as irradiation of meats and poultry. Ongoing research is determining the doses required to achieve meaningful reductions while preserving the quality of produce.

Howard et al. (1995) reported that 1.0 kGy reduced total aerobic bacteria and lactic acid bacteria in *pico de gallo*, a cold salad made of tomatoes, yellow onions, and jalapeño peppers, over six weeks of refrigerated storage, thereby extending the shelf-life. A dose of 1.0 kGy reduced total aerobic plate count and *L. monocytogenes* by ~4 logs (99.99%) on pre-cut bell pepper; however, on peppers stored at 15 or 10°C, the pathogen regrew to initial levels within 4 days, while pathogen levels remained low on peppers stored at 5°C (Farkas et al., 1997). Also in that study, spoilage bacteria were reduced by 5 logs on carrot cubes by 1.0 kGy. The authors concluded that irradiation, when combined with good manufacturing practices, could effectively reduce pathogen levels throughout the useful shelf-life of the produce.

Irradiation of diced celery (Prakash et al., 2000b) showed that 1.0 kGy reduced *E. coli* and *L. monocytogenes* to undetectable levels (>5 logs). Total aerobic counts following irradiation and three conventional treatments, i.e., acidification, blanching, or chlorination, showed that acidification reduced initial counts to a degree comparable to that of 1.0 kGy by the end of the storage period (22 days), but the aerobic population following conventional treatments had regrown to equal (acidification) or exceed (blanching and chlorination) the untreated controls; that of irradiated (0.5 and 1.0 kGy) celery remained significantly lower throughout the study.

The D_{10} values of 14 phytopathogenic fungi were evaluated in irradiated (0.5 to 1.5 kGy) mango pulp (El-Samahay et al., 2000). These ranged from 0.39 for *Aspergillus sydowii* and *A. ustus* to 1.11 for *Penicillium oxalicum*, confirming the relatively high radiation resistance of fungi. The D_{10} values were also determined in saline solution and demonstrated some variance with the data obtained in mango pulp. The more complex medium caused a decrease in D_{10} for some fungi, e.g., *A. ustus* (55%), *Scopulariopsis brevicaulis* (55%), and *A. sydowii* (76%), and an increased D_{10} for others, e.g., *P. brevicompactium* (147%), *A. flavus* (159%), and *P.*

oxicalum (179%). The highest dose (1.5 kGy) reduced total aerobic bacteria count by ~1.8 logs, a difference that persisted throughout the study (50 days). Shelf-life in irradiated (1.0 kGy) fruits was extended from 25 to 50 days in refrigerated storage. Although the interiors of the fruit used in this study were sampled and found to be free of contamination, the variable effect of fruit pulp on the radiation resistance of resident microorganisms is clearly demonstrated. Pulp represents a combination of plant cell contents with extracellular fluid found in the intercellular spaces; nevertheless, this observation should be considered in light of evidence of internalization of pathogenic bacteria into intercellular spaces (Takeuchi and Frank, 2000).

Several types of salad sprouts were inoculated with a cocktail of *E. coli* O157:H7 strains or *Salmonella* strains and irradiated by Rajkowski and Thayer (2000). Gamma radiation effectively reduced the bacterial load, with *E. coli* O157:H7 D_{10} values of 0.34, 0.27, and 0.26 on radish, alfalfa, and broccoli sprouts, respectively. *Salmonella* D_{10} on radish sprouts was 0.54 for isolates derived from meat sources and 0.46 for isolates derived from vegetable sources.

On dry spices (<10% moisture), D_{10} values for resident microorganisms were considerably higher than on fresh fruits and vegetables. Irradiated (10 kGy) paprika showed a 4-log reduction in total mesophilic bacteria, and similar reductions occurred in total *Enterobacteriaceae* and coliforms without significant loss of color (Nieto-Sandoval et al., 2000).

Irradiation may be combined with modified atmosphere packaging (MAP) to achieve a hurdle-type control of spoilage and pathogens. This approach has shown success with irradiated packaged meats (Thayer and Boyd, 2000). After irradiation under MAP, total aerobic counts were reduced ~3 logs on iceberg lettuce (Hagenmaier and Baker, 1997) and ~1.5 logs on romaine lettuce (Prakash et al., 2000a) by doses of 0.19 kGy and 0.35 kGy, respectively, differences that persisted during storage. Rate of spoilage and shelf-life were reported to be unchanged for iceberg lettuce, but the shelf-life of romaine lettuce was extended from 14 to 18 days to the length of the study, 22 days. MAP reduced total aerobic plate counts of shredded carrot irradiated (0.45 kGy) by ~2 logs, a difference that persisted throughout a 9-day storage evaluation (Hagenmaier and Baker, 1998).

Ionizing radiation is demonstrably able to penetrate into protected areas of produce (surface, subsurface, and interior) to damage bacteria; however, the extent to which a biofilm habitat may influence the radiation sensitivity of bacteria, either native nonpathogens or pathogenic contaminants, is not well understood. As the biofilm-participatory bacteria are suspended in a water-saturated exopolysaccharide matrix, they may be more at risk from oxygen and hydroxyl radicals generated during the irradiation process, thereby demonstrating an increased sensitivity similar to that seen in solution studies. However, the exact composition of the matrix is not yet known and may include radical scavenging compounds, which would reduce the efficacy of the irradiation (Sommers et al., 2002). Close proximity of different bacterial species with different levels of resistance may lead to postirradiation competition for resources that places additional pressure on radiation-injured bacteria; conversely, the matrix may provide for additional recovery from radiation injury by absorbing the radicals in the chemical bonds of the matrix or by protecting the bacteria from desiccation or competition. The microbial ecology of irradiated bio-

films is an area of active discussion, and development of competing hypotheses and will be an important area of research in the future.

Sensorial Properties of Irradiated Produce

Radiation-induced softening due to hydrolysis of pectins is a well-known phenomenon that is irradiation-dose dependent (Yu et al., 1996). Irradiated (1.0 kGy) celery was evaluated using analytical machinery and a trained sensory panel. The irradiated material had quantifiable texture, color, and aroma qualities equivalent or superior to untreated (controls), blanched, acidified, or chlorinated celery and maintained these qualities throughout the 22-day storage period of the study (Prakash et al., 2000b). Also, irradiated (1.0 kGy) celery had qualitative flavor, aroma quality, appearance, texture, and overall acceptability characteristics equal or superior to blanched, acidified, or chlorinated samples. The authors concluded that irradiation processing clearly results in a superior celery product vs. conventional processing means.

Iceberg lettuce (Hagenmaier and Baker, 1997) and romaine lettuce (Prakash et al., 2000a) were amenable to low-dose (<0.5 kGy) irradiation, with texture and shelf-life equal or superior to untreated controls. Strawberries softened following 1.0 or 2.0 kGy and chemical analysis showed an association with an increase in water-soluble pectin and a decrease in oxalate-soluble pectin (Yu et al., 1996). Cucumber pickles showed softening with increased dosage up to 1.0 kGy, but there was little effect on neutral sugars in the cell wall (Howard and Buescher, 1989). Potatoes showed softening following 0.2 kGy, and *in vitro* treatment of starches showed loss of viscosity postirradiation (Al-Kahtani et al., 2000).

Gibberellic acid–treated grapefruit tolerated 0.3 kGy with little loss of quality of the fruit, pulp, or juice, but after 0.6 kGy, pitting of the skin, softening of the fruit, and loss of juice quality rose to unacceptable levels (Miller and McDonald, 1996b). In a series of studies on blueberries, 0.5 kGy was identified as a dose that several cultivars would tolerate without loss of firmness and overall quality, but tolerance to 1.0 kGy was variable (Miller et al., 1994, 1995; Miller and McDonald, 1996a). Color, pH, decay rate, and other agronomic factors were reportedly not affected by dose level in these studies. Mango irradiated to 1.5 kGy were as acceptable to a sensory panel as nonirradiated controls immediately after treatment, based on odor, color, and overall appearance; irradiated fruit remained acceptable after 50 days of refrigerated storage, while nonirradiated controls became unacceptable after fewer than 30 days (El-Samahy et al., 2000).

Irradiation is known to oxidize a portion of the total ascorbic acid (vitamin C) to the dehydro form (Romani et al., 1963). Both of these forms of the vitamin are biologically active, suggesting minimal nutritional impact (Thayer, 1994); however, it is uncommon to see values for the dehydro form co-reported in analyses of ascorbic acid concentration.

Treatments up to 2.0 kGy did not produce any negative impact on radish sprouts (Rajkowski and Thayer, 2000). A dose of 1.0 kGy caused a 12% loss of ascorbic acid content in pre-cut bell pepper and a slight increase in beta-carotene content of cubed carrots, and the dose extended the shelf-life of both products (Farkas et al., 1997). Treatment with 1.0 kGy did not affect the sensorial properties of *pico de gallo*

but did result in loss of measurable ascorbic acid (Howard et al., 1995). The authors of that study discussed the high antioxidant concentration of the component vegetables (tomato, onion, jalapeño) as one possible mechanism for reducing the development of off-flavors and odors that can result from high-dose irradiation. Antioxidants can absorb oxygen and hydroxyl radicals formed during irradiation (Sommers et al., 2002) and may therefore be expected to protect plant tissues and cellular structures; this protection would presumably not extend to surface-bound microorganisms.

Although antioxidants added to nutrient solutions are known to protect suspended bacteria from the effects of ionizing radiation (Sommers et al., 2002), the role of natural antioxidants in determining radiation tolerance of fruits and vegetables has not been addressed directly. In reviews by Kader (1986) and Dupont et al. (1997), a variety of fruits and vegetables was grouped according to their tolerance for irradiation. More radiation tolerant products are, in some cases, also reported to be higher in antioxidant concentration, and lower radiation tolerance was similarly associated with low antioxidants (Cao et al., 1996; Wang et al., 1996); however, methodological differences in how the produce was evaluated in the various studies prevent definitive conclusions. Complications may arise through the localization or compartmentalization of the native antioxidants within the plant tissues, the effect of homogenization of tissues as part of the chemical analysis, heat degradation during sample preparation, or some other artifact of analysis.

A full discussion on methods to detect prior irradiation of fruits, vegetables, and juices is beyond the scope of this chapter. Many of the methods used for detection of irradiated meats, poultry, and shellfish may be adapted for these products; see reviews by Schreiber et al. (1993) and Sommers (chap. 14, this volume). Detection of radiation-induced hydrocarbons by gas chromatography ormass spectrometry has been successfully employed in irradiated (1.0 kGy) perilla (*Perilla frutescens*) seeds (Lee et al., 2000). Thermoluminescence has shown promise in detecting irradiation treatment of spices (Schreiber et al., 1993) and potatoes (Al-Kahtani et al., 2000). Measurement of electron paramagnetic resonance (EPR) is commonly used as a means of dosimetry, with alanine pellets as the dosimetry sample. EPR can detect prior irradiation of alfalfa (*Medicago sativa*) seeds (D.W. Thayer, personal communication). It should be noted that *ex post facto* dosimetry is difficult: although these and other detection methods may be able to indicate that a given product was irradiated, determining when the product was so treated, or the dose delivered, is unlikely (Diehl, 1995).

Influence of Plant Variety on Microbiological and Sensorial Response

Variety-specific response to irradiation is an important consideration, not only in determining the suitability of irradiation for treatment of modern varieties, but also in evaluating the applicability of information acquired in previous studies of irradiated produce. The most economically significant varieties of grains, vegetables, and fruits (and therefore the most likely subjects of irradiation research and implementation) change from year to year (Thelen, 1999; Wiersema and Leon, 1999). In studies with inoculated meat, pathogenic bacteria demonstrate varying radiation

sensitivity, depending on the specific type of meat evaluated (Thayer et al., 1995). Evidence suggests that, within a given crop plant species, the variety can also lead to varying radiation sensitivity.

Reviews of produce radiation sensitivity often combine different varieties under a single heading, e.g., "leafy vegetables" or "grape" (Kader, 1986; Dupont et al., 1997), a practice that does not recognize evidence of varietal differences. In separate studies of iceberg lettuce, *Lactuca sativa* var. Raleigh (Hagenmaier and Baker, 1997), and romaine lettuce, *L. sativa* var. Longifola (Prakash et al., 2000a), the texture, headspace gas composition, and microbiology of irradiated produce were assessed. Although irradiated (0.45 kGy) iceberg lettuce showed respiration 33% greater than nonirradiated controls, irradiated (0.35 kGy) romaine lettuce showed decreased respiration, based on oxygen content of the headspace gas after 1 day in storage. At relatively high doses (0.81 kGy), iceberg lettuce lost firmness, while at lower doses (0.2 and 0.5 kGy) the shear force of irradiated and nonirradiated leaves was virtually the same, with no apparent differences in texture. In contrast, loss of firmness of romaine lettuce irradiated at low doses (0.15 and 0.35 kGy) was readily apparent. Irradiation reduced the aerobic microbial population in iceberg lettuce (0.2 kGy) by approximately 3 log units, but in romaine lettuce irradiated at a higher dose (0.35 kGy), the reduction was only 1.5 log units.

Several varieties of sprouts were inoculated with a cocktail of *E. coli* O157:H7 strains and irradiated by Rajkowski and Thayer (2000). D_{10} values obtained on radish, alfalfa, and broccoli sprouts were 0.34, 0.27, and 0.26, respectively. Direct statistical comparisons were not reported, but the standard errors presented in that study were small, suggesting a variety-specific influence on the radiation resistance of the inoculated bacteria.

A comparison of the potato (*Solanum tuberosum*) varieties Ajax and Diamant (Al-Kahtani et al., 2000) showed differences in the postirradiation (0.20 kGy) viscosity of starches, whether isolated from irradiated tubers or irradiated *in vitro*. This difference was ascribed to unspecified varietal differences in chemical profile related to pectins, cellulose, and other tissue constituents. The blueberry (*Vaccinium* spp.) cultivars Brightwell and Tifblue did not differ in their response to gamma radiation, showing softening and similar loss of quality following 1.0 kGy but no negative impact after 0.5 kGy (Miller and McDonald, 1996a). However, although the flavor and texture of Sharpblue blueberries were considered acceptable following electron beam irradiation (1.0 kGy) (Miller et al., 1995), flavor and texture of Climax blueberries similarly irradiated (1.0 kGy) (Miller et al., 1994) declined significantly. Color, pH, decay rate, and other agronomic factors were not affected by dose level in these studies.

The nature of varietal specificity can be most clearly considered when data for different varieties are co-reported, but meaningful inferences are still possible when separate studies are considered. Care must be taken, however, when comparing the results from different single-variety studies conducted under widely disparate experimental conditions that perhaps include different means of irradiation, e.g., gamma vs. electron beam.

The mechanisms behind varietal specificity lie in as yet undefined differences in physiology and biochemistry related to irradiation response. Different levels of starch, pectin, and cellulose may lead to variable changes in viscosity, turgidity, or softening

as various chemical bonds are hydrolyzed (Howard and Buescher, 1989; Yu et al., 1996; Al-Kahtani et al., 2000). Levels of natural antioxidants may also play a role, by absorbing the oxygen and hydroxyl radicals produced during the irradiation process before they can damage tissue structures (Urbain, 1986). Also, the state of maturity of the vegetable at the time of irradiation may influence the efficacy of irradiation treatment and/or effects on product quality (Howard et al., 1995).

Differential microbial response to irradiation on different varieties may have multiple mechanisms. Varietal differences in surface topography, cuticle thickness, secondary metabolite profile, and other anatomical and biochemical factors are known to influence the development of plant pathogens (Agrios, 1988) as well as the efficacy of surface disinfectants applied to produce (Adams et al., 1989; Korber et al., 1997; Beuchat, 1998). By providing microhabitats with a unique microflora or biofilm community or a localized complement of secondary metabolites, plant surfaces with more varied topography may result in increased survival of pathogens in protective niches. Bacteria are known to penetrate into the tissues of lettuce (Takeuchi and Frank, 2000) and apple (Buchanan et al., 1999; Liao and Sapers, 2000), making them inaccessible to chemical disinfectants. The anatomical structures of most fruits and vegetables do not present a significant barrier to ionizing photons (gamma and x-ray), although the depth of penetration of ionizing electrons may be a factor for unusually thick or dense products (Table 13.1). A given mix of *Salmonella* isolates was shown to be approximately twice as resistant to gamma radiation when irradiated on seeds used for sprouting as when irradiated on the sprouts themselves (Thayer et al., 1999). The level of free water on a comparatively dry product such as seeds may have been a factor in this study; however, the authors ascribe the result to the presence of unidentified physiochemical factors associated with the topology of the seeds.

Fruits and vegetables present a complex substrate for studies of irradiation, with significant complicating factors of maturity, anatomy, topology, surface and internal chemistry, and native microflora, which may compete with or succor contaminating pathogenic bacteria. Furthermore, plant varieties wax and wane in commercial popularity and, as new combination products are created, e.g., salads, salsas, etc., the complexity of this interaction increases. Although a full discussion of packaging materials *vis–à-vis* irradiation is beyond the scope of this chapter, it should be noted that relatively few plastics are approved for use in irradiation processing out of concern for potential generation of volatiles and secondary food additives (Anonymous, 2000b). The effect of these factors, singly and in combination, on the efficacy of irradiation, as well as of chemical sanitizers and other treatments, will determine what doses will be most effective at eliminating contaminating bacteria and best tolerated by the fruit or vegetable product of interest.

IRRADIATION OF JUICES

The Case for Irradiation Processing

Conventional heat pasteurization is used for the overwhelming majority of juices sold in the U.S.; this pasteurization process results in the loss of essential oils and

other juice components, changing the flavor of the resulting juice. Although fresh, NTP juices are prized for premium flavor and aroma, these products have also been responsible for outbreaks of salmonellosis, enterohemorrhagic *E. coli* infection, hemolytic uremic syndrome, and other illnesses that have led to numerous hospitalizations and deaths (Luedtke and Powell, 2000). In response to these food-borne illnesses, the U.S. Food and Drug Administration has implemented a policy (Anonymous, 2001) requiring processors of NTP juices to implement control plans and technologies that will provide 5-log (99.999%) reductions in human pathogen load. These regulations will take effect for all processors by 2004; within this time frame, nonthermal antimicrobial measures must be brought to commercial standards of reliability and cost effectiveness if NTP juices are to be sold in the U.S.

A variety of nonthermal means of reducing the microbial load of NTP juices was recently discussed (Sizer and Balasubramaniam, 1999). These include pulsed electric fields, minimal thermal processing, high-pressure processing, and ultraviolet radiation. Regulatory approval for the use of ionizing radiation is currently being sought (NFPA, 2000). These technologies are being researched to determine the most applicable means of reducing the risk of pathogen contamination of NTP juices while preserving the flavor and aroma characteristics that allow these products to command premium prices.

Microbiology of Irradiated Juices

The majority of research efforts related to irradiation of juices has targeted spoilage organisms such as yeasts and molds rather than bacterial pathogens (Monk et al., 1994). Niemira (2001) reported a D_{10} value of 0.35 kGy for *S. enteritidis* irradiated in reconstituted orange juice. Buchanan et al. (1998) showed that irradiation (1.0 kGy) effectively inactivated *E. coli* O157:H7 in inoculated commercial apple juices, with D_{10} values of 0.12, 0.16, and 0.21 kGy for the three isolates tested. Niemira et al. (2001) found that the resistance of four *Salmonella* isolates irradiated in orange juice also varied, with D_{10} values of 0.71 kGy (*S. anatum*), 0.48 kGy (*S. newport*), 0.35 kGy (*S. infantis*), and 0.38 kGy (*S. stanley*). For the most resistant isolate (*S. anatum*), 3.5 kGy was indicated as the dose required to achieve a 5-log reduction. Similarly, *Salmonella hartford* was reduced to undetectable levels in irradiated (3.0 kGy) orange juice (Pickett et al., 2000). In that study, it was possible to recover the pathogen through selective enrichment.

The antimicrobial efficacy of irradiation is influenced by several factors. Native variation in pathogen resistance among different isolates has been noted (Buchanan et al., 1998; Niemira et al., 2001). Buchanan et al. (1998) showed that the radiation sensitivity of three strains of *E. coli* O157:H7 irradiated in apple juice was reduced 54 to 67% by previous growth of the cultures in acid environments. Also, the sensitivity of one test strain decreased in more turbid juices. This difference was ascribed to the antioxidant power of the suspended solids, although data for the antioxidant power of the juices were not presented. Although *L. monocytogenes* irradiated *in vitro* was increasingly protected in solutions of increasing antioxidant power (Sommers et al., 2001), *S. enteritidis* suspended in commercial citrus juices of varying composition (orange vs. orange/tangerine, extra pulp vs. pulp free, regular

vs. calcium enriched, etc.) and varying antioxidant power did not differ in D_{10} value following gamma irradiation (Niemira, 2001). The role of natural and artificial antioxidants is therefore an area to be addressed more fully.

Sensorial Properties of Irradiated Juices

As in whole fruits, irradiation oxidizes a portion of juice ascorbic acid to the dehydro form; as in fruits, this conversion is expected to have little nutritional impact (Romani et al., 1963; Thayer, 1994). In a wide-ranging study, Fetter et al. (1969) irradiated a variety of commercially pasteurized juices. Taste panel evaluation found a reduction of flavor quality in irradiated (5.0 kGy) grape juice, but similarly irradiated juices of orange, guava, tomato, red currant, black currant, apricot, peach, pear, and apple were not affected. Chachin and Ogata (1969) treated grape, apple, and orange juices with sterilizing (2 to 80 kGy) doses of gamma radiation. Loss of grape juice anthocyanin and orange juice beta carotene was evident after 10 kGy and was dose dependent up to 80 kGy. Browning was reported in apple juice after 5 kGy and ascorbic acid was reduced in apple and orange juices. Dehydroascorbic acid was not reported. Although 10 kGy reduced the acceptability of orange and apple juice, 0.01% propyl gallate reduced the loss of quality of orange juice thus irradiated.

In a later study, high-dose irradiation (10 kGy) of orange juice resulted in a similarly unacceptable degree of flavor degradation and browning; however, the addition of 0.1% sorbic acid before irradiation effectively eliminated loss of flavor and reduced browning (Thakur and Singh, 1993). Spoto et al. (1997) irradiated orange juice concentrate and determined the effect of storage time and temperature on juice quality and acceptability. In that study, the highest dose (up to 5.0 kGy) combined with 25°C storage resulted in loss of "orange" flavor and an increase in "bitter," "medicinal," and "cooked" ratings by sensory panelists. Lower dose (2.5 kGy) and cooler storage (0 or 5°C) were proposed as an acceptable processing regimen.

Niemira et al. (2001) found no evident changes in the appearance or aroma of reconstituted orange juice irradiated to 2.5 kGy. Pasteurized orange juice irradiated to doses as high as 5.0 kGy demonstrated little reduction in product color or concentration of key aroma and flavor compounds (X. Fan, personal communication). Unpasteurized apple cider irradiated to 3.0 kGy was identifiable, although not unacceptable, to sensory panelists evaluating aroma; similar results were obtained with reconstituted and fresh orange juices (B.A. Niemira and X. Fan, unpublished data). In contrast, Miller and McDonald (1996b) reported that the juice from irradiated grapefruit was acceptable after 0.3 kGy but declined in quality at 0.6 kGy. Pickett et al. (2000) reported increasing off-flavor in unpasteurized orange juice irradiated to 3.0 kGy, rendering the juice unpalatable.

Although research into the organoleptic qualities of irradiated juices continues, the factors that may lead to undesirable flavors, aromas, or changes in appearance are complex. While extensive studies into the effect of the variety and maturity of the source fruit on the radiation sensitivity of the resulting juice are lacking, it is known that juice composition is variety dependent (Huggart et al., 1975). In light of the evidence for varietal specificity in irradiated whole produce, the possibility of varietal specificity in juices with regard to negative irradiation effects cannot be

discounted. Temperature control during the irradiation process, as well as the possibility of ozone generation in the headspace gas of sealed containers, should also be carefully considered as potential secondary factors in juice irradiation.

CONCLUSIONS

With regard to food safety concerns, irradiation is, first and foremost, an antimicrobial treatment. As with other antimicrobial and preservation treatments such as chlorination, blanching, MAP, and ozonation, the specific details of how best to apply the treatment are critical. In the case of low-dose irradiation, many of these details are still being determined. A great many factors that influence what doses are necessary to inactivate bacteria while preserving produce and juice quality have yet to be explored in a systematic way, including:

1. Pathogens living in a biofilm habitat
2. Multicomponent salads and vegetable mixes
3. Varietal specificity in radiation tolerance and native microbial load
4. Internalization of pathogens; variation in concentrations of native antioxidants
5. Different combination protocols with other treatments in a hurdle approach
6. The specific type of irradiation employed, i.e., gamma vs. x-ray vs. e-beam
7. Other, as yet unidentified, factors

These factors will play a part in determining where, how, and whether fresh fruits, vegetables, and juices are irradiated. Irradiation holds a great deal of promise as a minimally invasive, zero-contact, postpackaging terminal control step. The application of the technology awaits the results of research to address the concepts and concerns presented in this chapter.

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